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TWENTY-FOURTH ANNUAL MEETING.

BALTIMORE, DECEMBER 27, 28, and 29, 1911.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

THE RELATION OF CALCIUM TO THE CARDIO-INHIBITORY FUNCTION OF THE VAGUS.

By H. H. Hagan and J. K. Ormond.

The experiments are in the main a confirmation of results obtained by Busquet and Pachon on the frog indicating that the presence of calcium in the perfusion fluid is essential to produce vagus inhibition of the heart. Slider terrapins, fresh-water turtles (snappers), and frogs were used. The effectiveness of vagus stimulation was studied when the perfusion fluid (0.7 per cent sodium chloride) was altered by the addition of varying amounts of calcium and potassium. The additional result is reported that a sodium-calcium solution too weak in calcium to restore vagus action will do so in the presence of a small amount of potassium chloride. In other words, the presence of potassium lowers the threshold value of the calcium necessary for vagus action. These results are explained on the assumption that calcium is necessary in the nerve apparatus for the conveyance of the vagus impulses to the heart muscles.

THE EFFECT OF ANÆSTHETICS ON PERMEABILITY.

By W. J. V. Osterhaut.

Experiments were performed to test the electrical conductivity of living tissues in various solutions. The results show conclusively that a great variety of ions readily penetrate living cells, and that this penetration may be markedly hindered or accelerated by the addition of various substances to the solution. The addition of anæsthetics, such as ether and chloroform, has a retarding effect on the penetration. It would seem, therefore, that these substances should retard all physiological processes which depend on the transport of ions through living tissues.

THE RÔLE OF PROTEINS IN GROWTH.

BY THOMAS B. OSBORNE AND LAFAYETTE B. MENDEL.

THE proteins satisfy several functions in the growing organism. A certain minimum is necessary for the maintenance represented by Rubner's "Abnutzungsquote." With an additional adequate energy supply any excess of protein beyond this maintenance requirement may, in the adult, experience temporary storage or be devoted to dynamogenic uses, but in the organism capable of development it may contribute to growth.

The perfection of a product containing the non-protein constituents of milk (protein-free milk) in a form adapted to the specific needs of growing rats has made it possible to examine the efficiency of individual proteins in respect to maintenance and growth respectively. The investigations have indicated the inadequacy of all protamines, namely, zein, gliadin, and hordein, in contrast with efficient proteins such as casein, lactalbumin, ovalbumin, edestin, glycinin, and glutenin, in promoting growth. Gliadin and hordein satisfy the needs of maintenance in young animals; zein does not. It will be noted that all of the inadequate proteins are deficient in two or more familiar amino-acid complexes ("Bausteine"). Details of these experiments are presented in Publication 156, Part II, Carnegie Institution of Washington (1911).

TEMPERATURE REGULATION IN THE WOODCHUCK (MARMOTTA MONAX).

BY SUTHERLAND SIMPSON.

DURING the last two years I have examined the rectal temperature of over fifty healthy, uninjured woodchucks caught in July, August, and early September in box-traps, and brought to the laboratory immediately after or within a day or two of their capture. The highest recorded was 106.2° F., and the lowest 92.4° F., with all grades between these limits. For example, in one batch of seven examined fresh from the field at the same hour the records were 106.2°, 104.5, 104.2, 101.4, 100.1, 97.8, 96.2. These animals therefore cannot be said ever to have a normal temperature in the sense that a homoiothermal animal has.

To test their power of heat regulation in a very cold and very warm environment, I placed six woodchucks, two rabbits, and one cat, each in a separate cage, in a room with a temperature of 20° F., and left them there for eight hours. At the end of that time the fall in the rectal temperature of the woodchucks was as follows: 4.5° F., 4.4, 3.7, 3.4, 2.8, and 2.2; one of the rabbits had lost 0.2° F., the other had gained 0.7° F., and the cat had lost 0.3° F.

On another occasion six woodchucks, with two rabbits as controls, were kept for four hours in a room with a temperature of about 108° F., and in that time the rise in the rectal temperature of the woodchucks was 2.3° F., 3.0, 3.7, 4.6, 10.2, and 13.2, and in the rabbits 1.5° F. and 2.6 respectively.

For the same animals the temperature also varies markedly at different times, as the following records will show :

Woodchuck	Aug. 26	Sept. 15	Oct. 13	Nov. 11	Nov. 27	Dec. 18
	°F.	°F.	°F.	°F.	°F.	°F.
I.	101.1	98.3	100.8	97.7	95.1	98.4
II	100.2	99.1	94.2	97.2	96.8	92.5
III	98.3	100.2	101.4	100.3	97.7	93.0
IV	96.9	100.4	94.8	. . .	100.8	97.2
V	101.1	98.9	100.9	. . .	99.1	101.2
VI	100.6	99.2	100.0	98.3	100.3	101.1

The animals from which the above records were obtained are kept in an enclosure out in the country in artificial burrows but quite unprotected from the weather. Food is placed within their reach every day and it regularly disappears, and on the date on which this is being written (December 26) they are still active and have not begun to hibernate. This would appear to show that the onset of hibernation is associated with a diminished food supply, and is not due entirely to a cyclical body change nor to the lowered temperature of the environment.

A COMPARISON OF THE DATA FROM TWO FASTS EACH EXCEEDING ONE HUNDRED DAYS IN LENGTH AND MADE UPON THE SAME SUBJECT.

By Paul E. Howe and P. B. Hawk.

Certain findings in an initial fast of one hundred and seventeen days in a 26 Kg. dog were contrasted with similar data obtained from the same animal in a subsequent fast; 700 c.c. of water was fed daily throughout each fast by means of a tube. During the interval between the fasts the animal was fed until it had regained its original body weight.

The disintegration of protein tissue, as measured by the total nitrogen output, was 7.1 per cent less during the second fast than during the first one hundred and four days of the first period of inanition. There was also a less rapid loss in weight during the second fast. This difference aggregated 10.3 per cent for the entire one hundred and four days, whereas if the second fifty-two-day period be compared, the difference is found to be 21.7 per cent. Data are also given for the distribution of nitrogen.

On the basis of these and other similar findings made in our laboratory we feel warranted in drawing the conclusion that through the medium of an initial fast an animal acquires a sort of resistance or immunity which enables it to more successfully resist the demands made upon it by a subsequent period of inanition.

MICROSCOPIC STUDIES ON LIVING SMOOTH-MUSCLE.

By Edward B. Meigs.

The experiments to be described are intended to throw light on the question whether smooth muscle cells decrease in volume during contraction.

In fixed preparations of smooth muscle the contracted cells have no greater diameter than the uncontracted cells except at the nuclear regions. The nuclei of the contracted cells are both shorter and thicker than those of the uncontracted cells, and the contracted cells are consequently bulged out in the nuclear regions.

If the fresh mesentery of Desmognathus fusca, one of the small urodelous amphibians, be examined in Ringer's solution, it is seen to contain smooth muscle cells, some of which have long narrow nuclei; and others, short thick nuclei. The cells with short thick nuclei are bulged out in the nuclear regions just as are the fixed contracted cells, and the diameters of the cell bodies at distances of ten micra from the ends of the nuclei are the same in the two kinds of cells.

Cross sections of frog's stomach muscle from 0.1 mm. to 0.2 mm. thick will live for half an hour or more in Ringer's solution or in Ringer's solution made slightly alkaline with sodium carbonate, and it may be shown that the muscle has a tendency to relax in the former solution and to contract in the latter. If such sections are examined microscopically after they have been for a few minutes in the Ringer and alkaline Ringer solutions respectively, it will be seen that the interstitial spaces are markedly larger in the (contracted) sections in alkaline Ringer. It may be shown that the muscle has no marked tendency to swell in the alkaline solution, and it must therefore be supposed that the extra fluid in the interstitial spaces of the sections treated with this fluid comes from the fibres.

Studies of fresh smooth muscle, therefore, confirm the view made probable by the results with fixed preparations that the cell bodies of the tissue decrease in volume during contraction.

THE CHEMICAL CONSTITUENTS OF THE ASH OF SMOOTH MUSCLE.

By L. A. Ryan and Edward B. Meigs.

The smooth muscle of the bull-frog's stomach has been analyzed for potassium, sodium, phosphorus, and chlorine by methods in general similar to those described by Katz in the Archiv für die gesammte

Physiologie, 1896, lxiii, pp. 1 *et seq.* Three determinations have been made of the potassium, sodium, and chlorine; four, of the phosphorus. The following are the amounts of the elements found in the different determinations and the averages, given as per cent of the fresh tissue: Potassium I, 0.306; II, 0.343; III, 0.346; average, 0.332: Sodium I, 0.051; II, 0.065; III, 0.080; average, 0.065: Phosphorus I, 0.128; II, 0.133; III, 0.146; IV, 0.149; average, 0.139: Chlorine I, 0.099; II, 0.120; III, 0.121; average, 0.117.

Parallel analyses of the ash of the striated muscle of the same frogs were made, and the results obtained were quite close to those reported by Katz for frog's striated muscle. The work indicates that smooth muscle contains somewhat less potassium and phosphorus and somewhat more sodium and chlorine than striated muscle, but the differences are much less marked than has sometimes been supposed.

The chemical work was supplemented with microscopic study of fixed and fresh samples of the tissue analyzed as "smooth muscle," and it was found that 70 per cent to 85 per cent of its volume was smooth muscle fibre; about 5 per cent, extraneous connective tissue; and the remainder, interstitial spaces between the muscle fibres.

THE ACTION OF THE DIGITALIS GROUP UPON THE HEART AND ITS SIMILARITY TO CARDIAC ANAPHYLAXIS.

By J. Auer.

In a previous communication dealing with anaphylaxis in the rabbit it was proven that acute death in this condition was due to a loss of irritability and contractility of the heart, and the incidental observation was made that digitalis preparations not only exerted no beneficial effect but seemed even to hasten the onset of death.

In following up this indication it was found that lethal doses of digitalis preparations (chiefly strophanthin, though fluid extract of digitalis, digitoxin, digitalein were also used) caused blood pressure and cardiac changes in the rabbit which were very similar to those observed in anaphylactic rabbits. The blood pressure in both conditions shows an initial slowing of the heart rate, followed later by a considerable increase in the rate, which is associated usually with a rise in the pressure and a marked diminution of the pulse amplitudes (membrane manometer). Following this, the blood pressure gradually falls, and the heart contractions become irregular and finally cease entirely.

If the heart is now examined, it will usually be found fairly dilated, especially on the right side, and the ventricles show no contraction or only very feeble attempts. Stimulation of the ventricles by mechanical or electrical stimuli causes no contraction, as a rule.

In addition to this similarity between the cardiac action of anaphylaxis and digitalis in rabbits, there is another point of correspondence which has not been described for digitalis, as far as I am aware. In rabbits which have succumbed to digitalis preparations or to acute anaphylaxis, the right ventricle shows a toughening of the muscular fibres when tested with the finger nail scraping the endocardial surface. In marked cases the right ventricular muscle near the auriculo-ventricular groove resists the scraping almost as if it were connective tissue. The left ventricle, however, is very soft, and muscle tissue is very easily scraped away.

These observations upon the functional and anatomical changes of the heart when subjected to anaphylaxis may perhaps throw some light upon the causation of the various kinds of heart block.

FURTHER STUDIES ON THE METABOLIC INFLUENCE OF ETHER ANÆSTHESIA.

By E. L. Ross and P. B. Hawk.

The study consisted primarily of an inquiry into the influence of diet and of a sub-normal body temperature upon post-anæsthetic glycosuria. The subjects used were dogs, and the anæsthesia was brought about either by means of the new "dehydrated" ether or by the ether customarily employed as an anæsthetic. Two methods of administration were employed — one a compressed air method in which the apparatus concerned was similar to that of Gwathmey; whereas in the other instance the ordinary "Cone" method was utilized. In a portion of the experiments a specially devised apparatus was employed to maintain the normal temperature of the animal during the anæsthesia period. In each instance this period was two hours in duration.

When fed diets principally of meat but containing from 3.3 to 4.1 gm. of carbohydrate per kilogram body weight, the dogs gave no evidence of post-anæsthetic glycosuria under any conditions, that is, with either type of anæsthetic, either method of administration, or after a pronounced lowering of the body temperature. When the carbohydrate portion of the diet was entirely replaced by meat, post-anæsthetic glycosuria was observed in every instance, irrespective of the character of the anæsthetic, the mode of its administration, or the course of the body temperature. All urines were examined qualitatively by means of copper and bismuth reduction tests and quantitatively by means of fermentation and polarization.

METABOLISM IN AN EXPERIMENTAL FEVER WITH SPECIAL REFERENCE TO THE CREATININE ELIMINATION.

By Victor C. Myers and G. O. Volovic.

Fever was induced in rabbits (ten experiments) by inoculation with the bacillus of hog cholera. Determination of total nitrogen, urea, ammonia, creatinine, creatine, chlorides, potassium and phosphates, together with the morning and evening temperature observations, were made in the urine during the fever period and a previous control period of four or more days. The creatine findings were of particular interest. The elimination of this substance during the fever was found to parallel very closely the body temperature, likewise the total nitrogen and urea, though the percentage of creatinine nitrogen in terms of total nitrogen dropped slightly at the height of the fever (3.8 to 3.3 per cent). The maximum temperature (about 42° C.) was always found to be accompanied by the highest creatinine elimination, the percentage increase over the normal elimination averaging 36 per cent during this period. The elimination of creatine did not always accompany the fever, but when present was generally observed following the crisis of the disease. The view is expressed that the increased creatinine elimination still represents the normal endogenous protein metabolism which is proceeding at an abnormal intensity due to the increased temperature, while the presence of creatine suggests the exhaustion of the normal glycogen store of energy, and perhaps measures the amount of abnormal endogenous protein metabolism.

RELATION OF THE LIVER TO REGENERATION OF FIBRINOGEN.

BY W. J. MEEK.[1]

IN the experiments reported an attempt was made to find the relation of the liver to fibrinogen formation by means of the Eck fistula. Dogs were used, and the fistulæ were made according to the method of Fischler and Schröder. The fibrinogen was determined as fibrin and also as fibrinogen by the method of Reye. While whipping out the fibrin, washings from previous clots were added to insure an excess of thrombin. Fibrinolysis was also guarded against.

The first point investigated was the rate of fibrinogen regeneration in a dog with the liver circulation intact. After partial defibrination accomplished by bleeding, whipping, and reinjection, fibrinogen was re-formed at a rapid rate. In some cases as much as 100 per cent increase was noted in three hours. After an Eck fistula and ligation of the portal vein, fibrinogen was still re-formed, but at a slower rate. After an Eck fistula, ligation of both portal vein and hepatic artery and partial defibrination, there was no longer regeneration, and the fibrinogen remaining in the blood began to rapidly disappear. Even without partial defibrination there was some evidence that fibrinogen content of the blood slightly decreases after extirpation of the liver by means of an Eck fistula and ligation of the portal vein and hepatic artery.

The simplest explanation of these observations would seem to be that the liver itself forms fibrinogen. The portal blood supply being largest, most of the constituents are taken from this source, but if the portal stream is diverted hepatic arterial blood is then used. Disappearance of the substance from the blood may speak for its having some kind of a *rôle* in general bodily metabolism. Another explanation of the observations might be that the liver conditions the formation of fibrinogen in some distant organ by means of a hormone or other stimulating mechanism.

A STUDY OF THE SECOND POSITIVE AND SECOND NEGATIVE WAVES OF THE VENOUS PULSE.

By E. M. Ewing and H. C. Jackson.

Simultaneous records of the contractions of the right auricle and ventricle, intraventricular pressure, subclavian pulse, and pulse of the vena cava were made in dogs.

With all possibility of arterial impact eliminated, a second positive wave, synchronous with the beginning of ventricular systole, appeared in the venous pulse tracing in all of the experiments. We conclude, therefore, that the second positive wave of the venous pulse originates in the auricle as result of the pushing up of the a-v valves during the period of rising tension in the ventricle.

The second positive wave comes to an end within 0.01 second of the opening of the semilunar valves, probably as the result of the downward movement of the a-v diaphragm and of the relaxation of the auricle.

That the second negative wave is more the result of the relaxation of the auricle itself than of the downward movement of the a-v diaphragm, is indicated by the fact that, with only the auricle contracting, there is a much greater fall in pressure than when only the ventricle is contracting.

The second negative wave is terminated by the third positive wave near the beginning of the plateau in the ventricular myogram. At this point the auricle is just completely relaxed, and, according to Roy and Adami's tracings, the downward movement of the a-v diaphragm has just ceased.

With strong auricular contractions the second positive wave occurred further down upon the first negative wave than in case of weak systoles. The greater fall in pressure in case of strong contractions is explained by the fact that more blood is eliminated than when the auricular systole is only of medium strength.

When the auricle only is contracting (fibrillation of the ventricle or heart block), there is a second positive auricular wave which occurs at precisely the same time as the normal second positive wave, which is supposedly due to ventricular systole. This second positive auricular wave coincides with, or augments, the normal second positive wave when the auricle and ventricle are contracting in normal sequence, but in cases of abnormally long a-v intervals it appears as an extra wave between the first and second positive waves. It is undoubtedly identical with Bard's "intersystolic wave," and as it is the more pronounced the stronger the auricular contraction and the more completely the auricle can empty itself into the ventricle, it is probably due to a recoil from the ventricle, together with a further pushing up of the a-v valves by the blood, which, as a result of auricular systole, has suddenly entered the ventricle and produced eddy currents behind the cusps of the valves.

HEART BLOCK PRODUCED BY COMPRESSING THE HEART NERVES OF LIMULUS POLYPHEMUS.

By Walter E. Garrey.

In the heart of Limulus polyphemus impulses arising in the ganglion on the posterior segments are conducted to the anterior segments by nerves only. By compressing these nerves with a clamp it was possible to duplicate the features of heart block as seen in the vertebrate heart, but modified by the peculiarities of the cardiac tissues of the limulus heart.

1. The impulses passing to the anterior segments were weakened in proportion to the degree of pressure on the nerves. Even after complete suppression of the impulses recovery could take place after decompression.

2. Weak impulses were blocked by less pressure than stronger impulses. By compressing the nerves a two to one rhythm was established between the posterior and anterior segments if the impulses

alternated in strength. Ganglionic impulses of different strengths were frequently arranged in regularly recurring groups. In such cases different degrees of compression gave partial blocks with different rhythms.

3. During severe compression and following decompression the passage of a single impulse, or of several impulses in some instances, established a refractory condition in the nerves. While this refractory stage lasted, the succeeding impulses were weakened or suppressed and partial block resulted.

4. Variations in the excitability of the myocardium were demonstrated which were frequently periodic in character. When the excitability was low, impulses weakened by clamping the nerves produced no contractions, although contractions occurred during the more excitable periods. In this way another type of partial block was produced.

BLOOD PRESSURE IN THE NORMAL UNANÆSTHETIZED ANIMAL UNDER VARIOUS CONDITIONS.

By Clyde Brooks.

The experiments reported were made on the wholly normal, quiescent, unanæsthetized animal employing the methods previously described (Heart, 1910, ii, p. 5).

With proper surgical technique and under ether anæsthesia the T-cannula was inserted early in the forenoon. Late in the afternoon the blood pressure was observed by connecting the side arm of the T-cannula to the mercury manometer.

Changes of the blood pressure following changes in the position of the body are in general characterized by quick readjustment to the normal pressure.

Section of one vagus was followed by slow respiration, with the pulse rate twice as rapid during inspiration as it was during expiration. The blood pressure rose during inspiration and fell during expiration. Section of both vagi resulted in phases of extremely high pressure and rapid pulse with cessation of respiration, followed by a phase of very low blood pressure with rapid weak heart beat and finally asphyxia with return of respiration. These phases alternated. In one instance the animal succumbed to the asphyxial stage.

A NOTE ON THE PHARMACOLOGICAL ACTION OF VANADIUM.

By D. E. Jackson.

When toxic doses of vanadium are administered to an animal, there are produced the ordinary gastro-intestinal symptoms common to all irritant metallic substances. Aside from these effects, however, the specific action of the metal in moderate doses is mainly exerted upon the vascular system and in the production of an increased intestinal peristalsis. There appears to be no stimulation whatever of the bulbar vasoconstrictor centre. Oncometric tracings of the kidney, spleen, and a loop of the intestines in an intact animal show a great diminution of their volumes. This action seems to be more profound and of much longer duration than that produced by epinephrine. It also differs widely from epinephrine in the extent of the rise in general blood pressure which it produces, for no increase in the size of the dose will produce more than about 50 or 75 mm. Hg, while the rise in general systemic blood pressure under epinephrine may be greatly in excess of this. Plethysmographic tracings of the leg volume in the normal intact (etherized) animal show a dilatation of the vessels of the limb. Under corresponding conditions epinephrine produces a contraction of the limb volume.

No specific action has been noted on the uterine musculature or upon the pupil. Probably certain smooth muscle fibres of the kidney and spleen are thrown into slight irregular contractions resembling sometimes the peristalsis which is set up in the intestines.

It appears that vanadium probably affects the vessels in a normal manner after very large doses of apocodeine. Ergotoxine has not yet been tried under similar conditions. Preparations are now in progress to carry out this experiment.

In perfusion experiments performed upon excised kidneys, spleens, and loops of the intestine it was found that the constriction of the vessels and diminished volume of the organs still appeared in the normal maximum intensity when vanadium was added to the perfusing solution. Therefore the action of the metal is peripheral and not central, as previously held. The volume of the amputated hind limb also decreases when vanadium is added to the solution with which it is perfused. This shows that in the intact animal the visceral vascular contraction displaces blood to the periphery in spite of a slight tendency to contraction in these vessels. This explains the sudden drop in temperature which has been observed following vanadium injections.

In most instances vanadium also differs widely from barium in the extent and character of its action.

THE EFFECT OF SPLANCHNIC STIMULATION ON MUSCULAR FATIGUE.

By W. B. Cannon and L. B. Nice.

The right tibialis anticus muscle is stimulated through its isolated nerve with single induction shocks, between 120 and 180 times per minute, until it is recording a uniform fatigue curve. Brief stimulation of the isolated left splanchnic nerve now results in a sharp rise and fall in the fatigue curve, followed by a slower rise and fall which may last from three to five minutes. The increase of muscular efficiency during the second rise may amount to 100 per cent. The sharp rise is due to increased blood pressure from constriction of the splanchnic area; it fails to appear if the gastro-intestinal tract is removed or the arteries are clamped. The slow rise is due to adrenal stimulation; it fails to appear when all adrenal vessels are ligated; and if the adrenal veins are clamped, it is delayed until the clamps are removed.

Increased adrenalin in the blood can conceivably increase the efficiency of skeletal muscle directly (experiments of Dessy and Grandis on isolated skeletal muscle), or by increasing blood pressure and thereby bettering the circulation. Injection of adrenalin in our experiments (2 c.c. of 1:100,000) increased the height of the fatigue curve for more than eighteen minutes after blood pressure returned to the original level.

Emotional increase of sugar and adrenalin in the blood, as already reported, accompanies fear and rage. These major excitements are likely to be attended in wild life by the necessity of running or fighting. Sugar would supply energy, and adrenalin would tend to obviate fatigue in the laboring muscles. The ability to continue during excitement prolonged efforts, ordinarily exhausting, can thus in part be explained.

OBSERVATIONS ON THE PHYSIOLOGY OF PURKINJE TISSUE.

By Joseph Erlanger.

The nerve elements and Purkinje fibres contained in the ventricular portion of the auriculo-ventricular conducting system are together designated Purkinje tissue. Petersen has shown that the false tendons in the left ventricle of the beef's heart alone may contain Purkinje tissue only, and at the same time be of sufficient size to be of use in a study of the physiology of Purkinje tissue. Consequently, experiments have been made on the false tendons of the perfused calf's heart. The experiments have demonstrated: (1) That false tendons are irritable, in the sense that when they are stimulated the ventricle may contract. This is in contrast to the results of stimulation of the true tendons. (2) That the false tendons are at least as irritable as the heart wall itself. (3) That they conduct the impulse apparently with equal facility in both directions. (4) That the rate of conduction in false tendons is at least 80 cm. per second.

The latent period of the heart plus the calcuated time required for transmission of the impulse from the auriculo-ventricular junction to the papillary muscle (0.06 second) is approximately equal to the spontaneous As-Vs interval (0.14 second). This suggests the thought that if there were a heart muscle synapse somewhere in the path of the impulse, the whole of the As-Vs interval could be accounted for.

Evidence has been obtained tending to indicate that the impulse is transmitted to the ventricular muscle at a point some distance removed from the point of insertion of the false tendons into the heart wall. In one experiment only did the false tendon give any evidence of being contractile; it was not possible to obtain records to verify this.

Histological examinations have demonstrated that most of the false tendons experimented upon have contained Purkinje tissue only. A few have contained heart muscle in addition. The presence of heart muscle does not seem to have modified the results.

A COMPARISON OF THE EFFECTS OF SUBCUTANEOUS AND INTRAMUSCULAR INJECTIONS OF ADRENALIN UPON THE PRODUCTION OF GLYCOSURIA.

By I. S. Kleiner and S. J. Meltzer.

In two series of experiments Auer and Meltzer have shown that various substances are better absorbed from muscular than from subcutaneous tissues; especially was this strikingly demonstrable for the action of adrenalin upon blood pressure. In a series of experiments we have tested the same point with regard to the production of glycosuria by adrenalin. The experiments were made on rabbits all of which had had no food for twenty-four hours previous to the experiments, but received by stomach tube 100 c.c. of water shortly before the injection of adrenalin. The urine was collected for twenty-four hours, although the elimination of sugar usually was finished long before. For intramuscular injection the lumbar muscles were selected; the subcutaneous injections were usually made in the lower part of the abdomen. The doses of adrenalin employed ranged between 0.3 c.c. and 1 c.c. of the 1:1000 solution. We shall not enter here upon details. It will suffice to say that for forty-nine pairs of animals the average amount of sugar eliminated was 0.73 gm. for the intramuscular and 1.20 gm. for the subcutaneous injections. There was a difference in favor of the subcutaneous injection in the averages of every tested dose of adrenalin. The difference, however, grew less with the decrease in the size of the dose. The largest difference was furnished by 0.7 or 0.8 c.c. of adrenalin. With all the doses there were some failures; that is, the injection of adrenalin brought forth no sugar. But here again, out of the forty-nine animals which received adrenalin intramuscularly there were thirteen failures, while in the forty-nine "subcutaneous" animals there were only four failures.

In connection with the foregoing we wish to mention briefly the result of another series of experiments. Meltzer has shown some time ago that strychnin is definitely more effective when a given dose is distributed over several places. We have therefore tried to inject subcutaneously a dose of 0.75 c.c. of adrenalin distributed over four different places. We made eight such experiments. The result was again seemingly paradoxical. Of the eight animals four had no sugar at all and the average for the other four was only 0.56 gm. The average amount of sugar for the same dose of adrenalin when injected in a single place was in our experiments 1.52 gm. We have here then again evidence that a method which favors absorption for other substances is unfavorable to the production of sugar by adrenalin.

There are, however, two established facts which make our results perhaps intelligible. One fact is that for the production of a rise of blood pressure the intravenous injection of adrenalin is the most favorable method, and the other is that for the production of glycosuria the intravenous method is apparently the least favorable. It is therefore possible that any method which favors the rapid absorption of adrenalin into the blood proves unfavorable to the production of glycosuria.

THE EFFECT OF ECK'S FISTULA ON THE FORMATION OF BILE.

By S. A. Matthews.

Dogs soon develop jaundice after ligation of the bile ducts and generally die in from one to two weeks. If in addition to the ligation of the bile ducts a biliary fistula be established, no jaundice will develop, but the animals generally suffer from digestive disturbances, and die in from four to six weeks in an extreme state of inanition.

If along with the ligation of the common bile duct and drainage of the gall bladder an Eck's fistula be established, bile will flow from the biliary fistula for from five to six days, after which it will cease and no icterus will develop.

If the common bile ducts be ligated without drainage, and after an interval of from six to ten days (after an intense icterus has developed) an Eck fistula be established, the jaundice will clear up and the dog may attain a fair condition of health in a few days.

No changes in the blood after the discontinuance of the bile formation have been noted (Voegtlein).

These experiments suggest the following conclusions: 1. The blood of the portal circulation may contain the substance or substances out of which the liver forms bile.

2. The portal blood may contain something which stimulates the liver cells to form bile or which may have a hormone-like action.

A METHOD OF STUDYING THE MOVEMENTS AND THE TONUS OF THE EMPTY DIGESTIVE TRACT BY THE X-RAY.

By A. J. Carlson.

Parathyroid tetany is usually accompanied by anorexia and vomiting, so that the bismuth subnitrate method offers special difficulties. In the course of an investigation of the condition of the digestive tract in parathyroid tetany we have found the following contrivances serviceable:

I. Catgut impregnated with silver nitrate and reduced by pyrogallic acid is rendered very opaque to the X-rays, and practically non-absorbable. Small pieces (0.5 to 1 cm.) of this gut are stitched to fundus, antrum, duodenum, etc. in such a manner as to form a triangle. The size of this triangle reveals active movements and large tonus changes. There is little if any tendency to encapsulate this modified catgut, at least for three weeks. The animals (cats) appear to suffer small or no inconvenience after the first two or three days.

II. Metallic wire. Small loops of platinum, silver, or lead wire fixed in the walls of the stomach and intestines, as under I, serve as well as the modified catgut, but seem to cause more irritation, leading in some cases to anorexia and vomiting.

The above methods have the disadvantage of revealing only limited portions of the digestive tract. Encircling large portions of the stomach and intestines with spirals of the modified catgut fails, for the reason that the catgut hardens and offers resistance to the movements, or does not follow the actual movements. To supplement method I in parathyroid tetany we have found the following contrivance serviceable:

III. A mixture of 10 per cent subcarbonate of bismuth in boiled starch is diluted to pass through a stomach tube, some meat extract added, and the whole given a 0.4 per cent acidity (HCl). This mass is fed through a stomach tube. Despite frequent interferences by vomiting, a combination of this procedure with the catgut method yields the best result in parathyroid tetany.

THE RESPIRATORY EFFECT OF ELECTRICAL STIMULATION OF THE CENTRAL END OF THE VAGUS NERVES IN DOGS UNDER INTRATRACHEAL INSUFFLATION.

By J. Auer and S. J. Meltzer.

Graphic tracings were obtained from a small rubber balloon placed in the œsophagus at a level with the lower part of the heart; the œsophagus was tied in the neck around the tube connecting the balloon with a Marey's writing tambour. The animals were usually under "half ether." In every animal we obtained during stimulation a complete expiratory standstill. When only one vagus was cut, the effects were limited; effective stimuli had to be fairly strong, for instance the inhibition of inspiration appeared only when the coil distance was about 150 mm. Moreover, with currents not much stronger than this, 80 or 60 mm. coil distance, the expiratory standstill did not last long, rapid inspiratory movements beginning soon. When both vagi were cut, the inhibitory response was much more prompt; definite expiratory arrest (inhibition) could be obtained, as a rule, already at 300 mm., and again, even with strong stimuli, at 60 or even 40 mm., there was a complete standstill during stimulation. There was no after-effect of stimulation with weaker stimuli and always a definite inspiratory after-effect with strong stimuli; while after less strong stimulation often a well-defined expiratory standstill, or a standstill which had to be considered as a compromise between expiration and inspiration preceded the definite inspiratory after-effects. These inspiratory after-effects were in some cases of very long duration; depending in most instances upon the strength of the foregoing stimulus and not so much upon the duration of the standstill. We must remember that with the insufflation method the necessary gas exchanges take place even during respiratory arrest.

The experiments show, that the vagus nerves contain expiratory and inspiratory fibres; that the inspiratory fibres require stronger stimuli than the expiratory; that when both kinds of fibres are stimulated simultaneously there is a compromise with a strong preponderance of expiration during stimulation, and of inspiration after stimulation; that the normal inspiratory stimuli from the moderately distended lung which pass through the intact vagus nerve antagonize in an effective degree the artificial stimulation of the cut vagus, and that finally the inspiratory after-effect is due to the foregoing stimulation of inspiratory nerve fibres, and not to a breaking through of the accumulated automatic inspiratory impulses within the respiratory centre.

POST-MORTEM ABSORPTION THROUGH LYMPHATIC VESSELS.

By Torald Sollmann and Paul J. Hanzlik.

In the course of our investigations on intestinal absorption it was found that considerable absorption may occur even in dead animals. The drugs were placed for about half an hour in ligated loops of intestine of dogs or cats at a varying period — up to several hours — after death. The intestine and contents were then assayed, and it was thus shown that a considerable proportion of the drug had disappeared: with phenol, up to 47 per cent; with sodium iodide, to 35 per cent: with alcohol, to 15 per cent.

We have not attempted to trace the mechanism of the post-mortem absorption of these drugs, but we have found that the post-mortem absorption of potassium ferrocyanide occurs exclusively by the lymphatic trunks. This applies to intramuscular as well as to intestinal injections. The ferrocyanide can be demonstrated in the lymph vessels to the next lymph gland, but not beyond. This indicates the existence of a post-mortem lymph flow, which can usually be demonstrated six hours after death, and occasionally even for forty-eight hours. It is independent of gravity or injection pressure, but may possibly be due to osmosis, since the ferrocyanide solution was hypertonic (10 per cent). The ferrocyanide could not be demonstrated in the blood vessels, so that the absorption is highly selective.

ON THE CROSSING OF THE RESPIRATORY IMPULSE AT THE LEVEL OF THE PHRENIC NUCLEI.

By W. T. Porter and Abbie H. Turner.

A recent study in this Journal affirms the well-known fact that the phrenic nerve in the cat and dog contains afferent fibres and suggests that the crossing of the respiratory impulse from the normal to the hemisected side of the spinal cord on section of the phrenic nerve on the normal side[1] is due to an afferent impulse set up when the phrenic nerve is stimulated by the section.

The crossing just mentioned was discovered in the rabbit. Upon stimulation of the central end of the phrenic nerve in the opened chest of the rabbit no reflex contractions of the diaphragm can be observed either by the unaided eye or in a graphic record. In the rabbit, therefore, the suggested explanation will not serve.

The essence of this explanation is that the respiratory impulse crosses the spinal cord because of the stimulus of cutting one phrenic nerve. But in the cat, in which the stimulation of the central end of the phrenic nerve does give rise to reflex contractions of the diaphragm, crossing takes place when the nerve, at the level of the heart, is divided physiologically, without stimulation, by freezing on a tube in which liquid carbon dioxide is evaporating.

The hypothesis of 1895 should therefore stand.

A METHOD FOR THE STUDY OF THE VASOMOTOR NERVES OF THE HEART AND OTHER ORGANS.

By W. T. Porter.

A large and a small reservoir filled with a blood mixture are placed 120 cm. above the animal. Tubes *A* and *B* (Fig. 1) from each reservoir unite in a single tube which leads through a normal temperature water jacket to a cannula in the ramus descendens of the left coronary artery of the dog's heart. As soon as the cannula is in position, the descendens is fed from *B*, and the suspected nerve laid upon electrodes. The stopcocks are now turned and the artery is fed from *A* instead of *B*. As the blood sinks in *A*, a float descends. The float pulls a fine wire which bends over two pulleys and thus lifts the glass thread *T*, the lower end of which writes upon a kymograph. The heart-beat and the carotid blood pressure are recorded by a membrane manometer.

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[1] Journal of physiology, 1895, xvii, p. 455.

If vasomotor fibres are present in the nerve, their stimulation narrows the artery, obstructs the outflow from *A*, retards the descending float, and bends the written line out of its unobstructed course. The apparatus may be empirically graduated, and the change in the circulation measured quantitatively.

This method leaves the animal intact except that defibrinated blood passes through the artery under investigation. The method avoids the errors and difficulties incident to the measurement of perfused blood as it escapes finally from the veins. Although devised for the investigation of the vasomotor nerves, the method has a wider value, as in the study of the relation between blood supply and muscular work, now in process in the writer's laboratory.

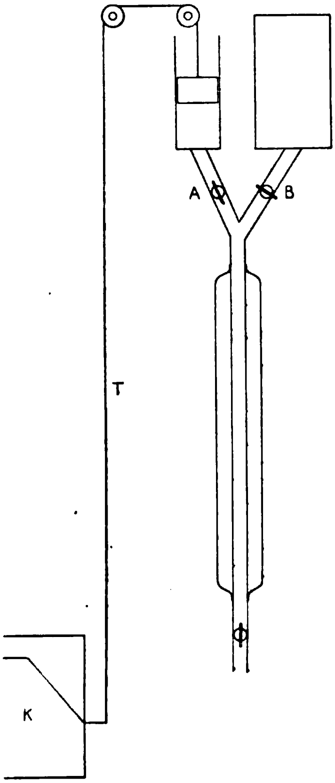

FIGURE 1.

INHIBITION OF RESPIRATION BY DISTENTION OF THE LUNGS OF DOGS UNDER INTRATRACHEAL INSUFFLATION.

BY J. AUER AND S. J. MELTZER.

HERING and Breuer's well-known observation that the distention of the lungs causes an inhibition of respiration was made by clamping the trachea either at the height of an inspiration or after an artificial inflation of the lungs. In these experiments the normal ventilation ceased during the clamping and the CO_2 content of the lungs and of the blood increased, which gave rise to respiratory efforts.

In dogs under intratracheal insufflation we were able to cause a long-lasting expiratory standstill by greatly increasing the pressure of the ingoing air stream. With the proper proportion between tube and trachea an increase of the pressure to 60 or 80 mm. causes a complete standstill in expiration which may last a minute and longer. On the release of the pressure the inspirations return somewhat gradually in most cases; in two or three instances, with very high pressures, there was even a definite short expiratory after-effect. It must be remembered that in our method the ventilation continues effectively even during complete respiratory standstill. When both vagi are cut, no standstill can usually be obtained by raising the pressure.

THE HOURLY CHEMICAL AND ENERGY TRANSFORMATIONS IN THE DOG, AFTER GIVING A LARGE QUANTITY OF MEAT.

By H. B. Williams, J. A. Riche, and Graham Lusk.

A calorimeter of the Atwater-Rosa type, constructed by Dr. Williams, is capable of measuring with great accuracy the heat of combustion of alcohol and the oxygen absorbed and the carbonic acid produced during alcohol combustion, in periods of one hour each.

A dog fed 700 gm. of meat at noon of the previous day was placed in the calorimeter between 10 and 11 A. M., and his metabolism measured. At 12 M. the animal was given 1200 gm. of meat and placed in the apparatus again. The heat production and other factors of metabolism were determined during hourly periods for twenty hours.

1. It was found that the direct and the indirect calorimetry agreed perfectly.

2. It was found that the heat production rose largely, and that this increase in heat production was proportional to the nitrogen eliminated in the urine, and was in no way proportional to the quantity of material present in the intestine.

3. It was found that the carbon which was retained from the protein

ingested must have been retained in the form of glycogen, since the absorption of oxygen during the different periods corresponded exactly with this assumption, whereas, if the carbon had been retained in the form of fat, the oxygen absorption would have been 10 per cent less than that found.

Further experiments have shown that glutamic acid added to a standard diet does not increase the heat production in any way.

THE EFFECT OF THE REMOVAL OF THE HEART UPON THE APPEARANCE OF CONVULSIONS IN FROGS.

By Thos. S. Githens and S. J. Meltzer.

It was reported in previous communications by one of us (M.) that an injection of morphin in cardiectomized frogs is capable of producing convulsions in forty to fifty minutes. It was found later that this result could not be obtained in the months of April and May; frogs usually died too soon after the removal of the heart to permit the development of convulsions. In the fall of last year we obtained again positive results, expecially so during the cold spells in the month of December. In a new series of experiments, morphin was injected first and the heart removed later at different intervals ranging between a few minutes and four hours. Practically all frogs which received a sufficient dose of morphin — between 0.2 to 0.5 mg. per gm. frog — had sooner or later definite convulsions, while the control experiments remained free.

THE EFFECT OF STIMULATION OF THE PERIPHERAL END OF THE SPLANCHNIC NERVES UPON THE PUPIL.

By Don R. Joseph and S. J. Meltzer.

In this study one superior cervical ganglion was removed from rabbits, and several days later the peripheral end of one splanchnic nerve (or of both nerves alternately) was stimulated electrically. Such a stimulation resulted, in a great majority of cases, in a variable but unmistakable dilatation of the pupil on the ganglion free side, while the pupil on the normal side remained practically unchanged. The dilatation usually lasted but a few minutes and then gradually disappeared.

Our interpretation of the result (which was also the working hypothesis) is that by the stimulation an amount of the secretion of the adrenals was thrown into the circulation sufficient to produce a dilatation of the pupil on the ganglion free side. After removal of a superior cervical ganglion, the pupil on that side is much more sensitive than normally to the action of adrenalin (S. J. Meltzer and C. M. Auer).

THE BLOOD PRESSURE FALL PRODUCED BY TRACTION ON THE CAROTID ARTERY.

By E. D. Brown and T. Sollmann.

Traction on the cephalic end of the common carotid artery in the neck causes a marked fall of blood pressure with uniform constancy in all animals investigated (dogs, cats, and rabbits).

There is at the same time a slight disturbance of respiration, which is governed largely by the degree of anæsthesia.

The phenomenon is not materially affected by division of the vagi, cervical sympathetics, and depressor nerves.

Observations made by means of the cardiomyograph, plethysmograph, oncometer, and by clamping the aorta show the reaction is purely of cardiac origin. It was found that the depressor, sympathetic vagus, and superior laryngeal nerves are not connected with the reflex either as afferent or efferent paths.

The traction fall occurs fully after section of both accelerators, after excision of both inferior cervical ganglia, after excision of both stellate ganglia, and after division of the splanchnics.

The reaction is transmitted through the internal carotid artery to the carotid plexus, whose nerve fibres probably constitute the afferent path.

STUDIES ON THE CONVULSIVE REFLEX PRODUCED BY STRYCHNINE.

By H. T. Mostrom and H. McGuigan.

A. Habit. — From two series of experiments on frogs in which strychnine was injected at intervals varying from one to four weeks an increased susceptibility to strychnine was developed. The average time of onset of spasms was reduced from thirty and one-fourth to thirteen and one-half minutes in one series of twelve frogs, and in another series of twelve it was reduced from twenty-six to ten and one-half minutes.

B. As modified by adrenalin. — From experiments on the exposed heart of frogs Falta and Jocovic assert that adrenalin is a powerful antidote to strychnine. Exener reports cases where guinea pigs and rabbits after intraperitoneal injection of adrenalin tolerate much larger doses of strychnine. Our experiments corroborated those of Falta and Jocovic on the heart only and were directly opposed to those of Exener. We find that after adrenalin the strychnine spasms appear more quickly than under strychnine alone, in spite of the fact that adrenalin in itself is markedly depressant to animals.

THE ABSORPTION OF FAT BY THE SALMON STOMACH, PRELIMINARY NOTICE.[1]

By Charles W. Greene.

During the summer just closed I was able to demonstrate that fat is absorbed by the stomach of the young king salmon. This demonstration was made by means of the method of staining fat with scarlet red, a modification of the Herxheimer procedure.

Young salmon were fed with olive oil by the method of rectal injection. After various lengths of time the fish were killed and the tissues examined either fresh or after fixation in 10 per cent formalin.

The salmon stomach has the usual two divisions, cardiac and pyloric. The cardiac division is provided with peptic glands of the ordinary fish type. The pyloric division is covered with an epithelial coat which is deeply folded and has pits here and there, but which does not possess definite tubular glands as well developed as in the mammalia.

Fat absorption from the cardiac region. — After fat feeding the cardiac division of the stomach shows an unusual quantity of fat droplets in the superficial epithelium. These droplets vary in size from those scarcely discernible with the 1/12 oil immersion lens up to those as much as two micra in diameter. In the earlier stages the fat is crowded in extremely fine droplets into the ends of the superficial epithelial cells. In the later stages relatively larger droplets of fat are seen to be distributed along the length of the cell, especially just above and just below the nucleus.

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[1] Abstract published by permission of the U. S. Commissioner of Fisheries.

Fat absorption from the pyloric region.—The superficial epithelial coat in the pyloric division of the stomach is more uniform in character, and there is little differentiation between it and the cells lining the deeper crypts. In my more successful fat-feeding experiments these superficial cells are simply crowded with fat. The loading extends from the free surface of the epithelial cell to its base, and the boundaries of adjacent cells are often obliterated by the excess of stained fat present. Following the cells down into the crypts, the amount of fat present diminishes. In some crypts the bottom cells have no fat at all, while the others have a liberal sprinkling of fat droplets. In the pyloric epithelium the fat droplets average larger than those in the cardiac end, yet they are much smaller in size than the drops of absorption fat in the epithelium of the intestinal region of the same specimen.

ABSORPTION OF FAT BY THE MAMMALIAN STOMACH.

By Charles W. Greene and William F. Skaer (by invitation).

Using a method of staining intracellular fat, Bell's modification of Herxheimer's alkaline alcoholic solution of scarlet red, we have been able to demonstrate conclusively that fat is absorbed by the mammalian stomach.

Our observations have been made on the laboratory mammals, — the dog, cat, and rat. In each we have found evidence that fat is absorbed through the lining mucous membrane of the stomach. This absorption takes place in all the regions of the stomach we have examined except through the corneous epithelium which lines a portion of the rat's stomach.

The absorption of fat in the cardiac end of the stomach.—Following the feeding of fat, we have observed that the superficial epithelial coat of the cardiac end of the stomach contains a heavy loading of fat. This fat is greatest in amount in those portions of the epithelium which border on the cavity of the stomach, but it is also present in the lining of the crypts as deep as the neck cells.

The fat is within the epithelial cells, but its distribution within the cell depends upon the time the sample is taken after feeding of fat. In the young animal the epithelial cells are loaded with fat in one hour, as shown on a four-day-old puppy, and in all animals an increased quantity of fat within the cells can be demonstrated within a very short time after fat feeding. Occasionally when absorption was especially rapid we have found numerous fine droplets of fat in the tissue spaces of the cardiac mucosa.

The absorption of fat from the pyloric region of the stomach. — In the mammals in our experiments, as in the salmon in the work reported by one of us, we have found that there is abundant absorption of fat in the pyloric division of the stomach. The superficial epithelial cells of the pyloric mucosa show the same stages of fat absorption which have just been described for the cardiac region. There is this difference, however, the fat is present in much greater amount and in relatively larger droplets. In an average stage of absorption the pyloric superficial epithelial cells will be crowded from apex to base to such an extent that one can scarcely distinguish the cell boundaries or the nucleus, even with a favorable counterstain. In the pyloric portion of the stomach the fat is found in the cells located much deeper in the mouths of the crypts than in the cardiac end.

THE PLACE OF RETENTION OR RECONJUGATION OF THE AMINO ACIDS IN THE BODY.

By Albert Woelfel.

If the mediation of either the liver or the intestinal mucosa is necessary to the animal organism for the regeneration of required albumen out of the split products furnished by the digestive processes in the alimentary tract, there ought, after a shunting of either of these organs out of the circulation and the introduction of amino acids into the system, to be a corresponding increase of amino nitrogen, or of ammonia, in the blood and the urine.

Dogs, operated on a few days previously for Eck-fistulæ, had their hepatic arteries tied and then solutions of amino acids, prepared by hydrolysis with enzymes, introduced into their washed-out intestines; two hours were allowed for absorption. All of the urine excreted was then collected and the animals bled completely. The blood was defibrinated and centrifuged, and the serum thus obtained had its albumen removed and was otherwise prepared for formol titration (Sörensen) to test it for an increase in its amino nitrogen content over normal serum. The ammonia and the amino nitrogen in the urines were determined.

The small increases of amino nitrogen in the blood and the small increases of formol titratable nitrogen in the urines were far from commensurate with the amounts of amino acid shown by determination to have been absorbed by the intestine.

Dogs with cœliac axis and mesentery arteries ligated show essentially the same relations in their blood and urines after known amounts of amino acid have been slowly injected intravenously; this is, however, also true if amino acids prepared by acid hydrolysis are injected.

That the amino acids are not removed from the blood with the fibrin in the defibrination, or that some constituent of the plasma or the blood corpuscles are not responsible for their disappearance was shown by control determination.

Tissues other than the liver intestinal mucosa, and blood cells can take up amino acids from blood plasma. Since the tissues in general seem to have such an avidity for amino acids, it is fair to suppose that in them the amino acids can be utilized for the regeneration of albumens.

THE ABSORPTION OF FAT IN THE SALMON MUSCULAR TISSUE AND ITS RESORPTION DURING THE MIGRATION FAST.[1]

By Charles W. Greene.

The king salmon stores large quantities of fat in its tissues during its life in the ocean. It ceases to feed when it enters the fresh waters of the rivers in the journey to the spawning ground. In the Columbia River those salmon caught at the lowest point at the mouth of the river have the greatest amount of stored fat.

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[1] Abstract published by permission of the U. S. Commissioner of Fisheries.

The fat is stored chiefly in the muscles; these are of three classes, namely, the dark muscle, the great lateral pink muscle, and the small muscles of the fins and head. The fat in the dark muscle is stored in large masses chiefly within the fibres, but to some extent between the fibres. In the pink muscle which represents the greatest mass the fat is wholly between the fibres up to the time when the salmon stop feeding. In the constantly active fin muscles there is only a slight amount of stored fat which is chiefly intermuscular.

From the time the salmon stop feeding until their death after spawning the quantity of stored fat gradually diminishes. It is never wholly consumed, even in fish taken after natural death. In the dark muscle the fat is gradually eliminated both from the inter- and intra-fibrous regions. It never wholly disappears from the substance of the muscle fibre, but is greatly reduced at the dying stage. In the lateral pink muscle the inter-fibrous fat is gradually removed during the migration period and has practically disappeared at the time of death.

An observation of especial interest consists in the fact that as soon as the fish enter fresh water at the mouths of the river a relatively large quantity of extremely finely divided fat makes its appearance within the muscle fibres. The fat is somewhat greater in amount and the droplets are slightly larger in the smallest fibres. This intra-fibrous fat is present in all specimens at all stages of the migration journey. Its quantity is remarkably uniform. In fish from the spawning grounds which are approaching the spawning period this intra-muscular fat begins to diminish in quantity. At the time of death, however, considerable quantities are still present in the smallest fibres, though it has completely disappeared in the largest fibres.

It seems evident that fat is thrown into the fibres of the great lateral muscle and kept there in strikingly uniform quantity and amount during the entire migration journey. It is suggested that this fat is utilized by the muscle as the source of the energy expended during the migration fast.

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CONTRIBUTIONS TO OUR KNOWLEDGE OF THE ACTION OF SODIUM AND CALCIUM UPON THE DIRECT AND INDIRECT IRRITABILITY OF THE MUSCLES OF THE FROG.

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INTRODUCTION.

IRRITABILITY of muscle tissue means its contractility. By an action upon the irritability of muscle is meant an increase or decrease of its contractility. Contraction is a readily recognizable phenomenon, and a study of the action of any substance upon this phenomenon ought to yield, we should expect, incontrovertible results. The numerous investigations of the last two or three decades dealing with the nature of the action of sodium and calcium upon the irritability of contractile tissue led nevertheless to practically diametrically opposite conceptions. While, for instance, Howell[1] and his pupils were inclined to look upon calcium as the factor which increases the irritability, ascribing to sodium chiefly the task of taking charge of the osmotic equilibrium, Loeb [2] and his followers, on the contrary, formulated the theory that the irritability increases with the rise of the value of the quotient Na/Ca, that is, the irritability of the contractile tissue grows with the increase of sodium or the decrease of calcium. It seems to us that in the path of these studies there are a variety of difficulties which heretofore have not been sufficiently taken into account, and which have to be surmounted before attempting to formulate a general law regarding the action of the mentioned inorganic agents upon the muscle irritability. In the first place contractility is not, as it seems to be tacitly assumed, a simple phenomenon; on the contrary, it is manifested by a variety of features, each of which may respond differently to the action of some agents. There is, for instance, the length of time during which a muscle may continue to respond with a contraction to a definite stimulus; under the influence of one substance this period may be lengthened, and under the influence of another, it may be shortened. Then there is the intensity of the minimal stimulus which causes a perceptible contraction — the threshold stimulus, which one substance may heighten and another lower.

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[1] HOWELL: This journal, 1899, ii, p. 47; 1902, vi, p. 181.

[2] See LOEB: Über physiologische Ionenwirkung, Handbuch der Biochemie, 1909, Bd. ii, 1 Hälfte, p. 104. See also the extensive bibliography in ROBERTSON, Ergebnisse der Physiologie, 1910.

Irritability may also be measured by the strength of the contraction (or the height of the graphic tracing), or by its duration, or by the extent of the contraction remainder. Or we may gauge the irritability of the muscle by the twitchings which one substance may inaugurate or increase and which another may decrease or inhibit. Now all these manifestations of contractility are separate phenomena, some of which are not only independent of one another, but run definitely in opposite directions. Take, for instance, the phenomena of the twitchings of muscle and the length of time during which the muscle is capable of responding to stimulation. Ringer,[3] who was practically the first one to discover that frog muscles twitch when bathed in a solution of pure sodium chlorid, observed at the same time that the addition of calcium to the solution abolishes the twitchings. It was the generalization gained from such observations which gave rise later to the conclusion that sodium increases and calcium decreases muscle irritability. We may, however, arrive at the opposite conclusion when the actions of these substances are considered from the point of view of their effect upon the duration of the responsiveness of the muscles to stimulation. According to Ringer,[4] frog muscles continue to respond with contractions to stimulations for twenty-seven hours, if they are kept in a solution of pure sodium chlorid; but they continue to react to stimulation as long as 40 hours, if the solution contains also some lime. This difference is even more striking when the muscles are continually excited with a short stimulus every two seconds. In sodium chlorid alone the muscles stop contracting after an hour and a half or after two hours; if the solution, however, contains also some calcium, the responsiveness continues for six or eight hours. That is, in a pure solution of sodium chlorid muscles fatigue nearly four times sooner than in a solution which in addition to sodium chlorid contains also calcium. That certainly looks as if it is the calcium which keeps up the irritability.

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[3] Ringer: Journal of physiology, 1886, vii, p. 291.

[4] Ringer: *Ibid.*, 1887, viii, p. 288.

Under these circumstances we need not wonder at the fact that different investigators arrived at diametrically opposite conclusions with reference to the action of sodium and calcium upon the irritability of muscle; it all depends upon which of the manifestations of contractility has been taken as a criterion of the irritability. In this connection it is instructive to glance at the subjects of investigations which formed the starting points for the divergent views. As is well known, Loeb's[5] starting point in our line of investigation was his extensive studies of the phenomenon of the twitchings of frog muscles when bathed in solutions of sodium salts and the inhibition of these twitchings by calcium salts and various other salts the kations of which are chemically related to calcium. Howell's[6] investigations upon the action of calcium began with his studies upon the causation of the heart beat and were based upon, and stimulated by, the observations of Ringer, that the frog's heart beats much longer in a solution of sodium chlorid containing calcium than in a solution of pure sodium chlorid, and, further, that the systolic tops as well as the systolic descents of perfused hearts became prolonged, as soon as lime was added to the solution of sodium chlorid. Here are manifestations of contractility which undoubtedly increase under the influence of calcium.

Another difficulty in the way of drawing general conclusions is the fact that for the same manifestation of contractility we may find that different muscle tissues respond differently to one and the same substance. The augmenting action of calcium, which Ringer has described, was observed, as stated above, on the muscular tissue of the frog's heart. Soon after Ringer's publication, Brunton[7] and Cash reported that the gastrocnemius of the frog also responds to calcium with a prolonged contraction. However, in a further study of the subject, Ringer himself pointed out emphatically that experience gained on the heart muscle cannot be applied to skeletal muscles; here calcium often inhibits such manifestations of contractility as in the heart muscle are augmented by it. When, for instance, a skeletal muscle remains twenty-four hours and longer in saline, the contraction brought on by a stimulus, and the period of relaxation of such a contraction, are often greatly prolonged; the addition of calcium, however, accelerates the contraction as well as the relaxation — which is just the reverse of the action of calcium upon heart muscle.

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[5] Loeb: Fick's Festschrift, Braunschweig, 1899, p. 101. See also Loeb, Studies in general physiology, Chicago, 1905, p. 518.

[6] Howell and Cooke: Journal of physiology, 1893, xiv, p. 198; and Howell: This journal, 1899, ii, p. 47.

Furthermore, even for the same kind of tissue and for the same category of manifestations of contractility, observations made on one variety of animals are not applicable, without a special test, to another variety of animals, even if both varieties are closely related within the domain of the same species. This is well illustrated in the experiments of Loeb with two varieties of hydro-medusæ. When the nerve ring of gonionemus, a medusa which abounds in Woods Hole, is removed and the centre returned to sea water, it remains perfectly quiet. If the centre be transferred to a pure solution of 5/8 N NaCl, which is isotonic with sea water, it starts beating rhythmically. These movements, however, cease as soon as some calcium is added to the solution of sodium chlorid.[8] This fact is in complete harmony with the behavior of the two ions towards the production of twitchings in frog muscles. However, Loeb found that the centre of the hydro-medusa polyorchis,[9] a medusa found in the Bay of San Francisco, does not beat in a pure solution of sodium chlorid isotonic with sea water, while the rhythmical swimming movements set in as soon as calcium is added to that solution. Here the relations are just the reverse of that observed in gonionemus and in frog muscles; the contractility seems to decrease with the increase of the quotient Na/Ca.

We may mention here another form of irritability which seems to increase in inverse proportion to the quotient Na/Ca; it is the so-called indirect muscle irritability. It was found by Carslaw, Locke and others that by perfusing muscles with, or bathing them in, sodium chlorid they lose their ability to respond with a contraction to a stimulation of their motor nerves. Locke discovered, however, that by the addition of a small amount of calcium, the lost indirect irritability is restored quite rapidly. Here again is an instance in which the (indirect) irritability is reduced by sodium and increased by calcium.

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[7] Brunton and Cash: Proceedings of the Royal Society, 1883, xxxv, p. 63.

[8] Loeb: This journal, 1900, iii, p. 383.

[9] Loeb: Journal of biological chemistry, 1905–1906, i, p. 427.

To recapitulate briefly: The phenomenon of contractility consists of a variety of manifestations some of which are augmented by sodium and depressed by calcium, while others are affected just reversely, they are depressed by sodium and augmented by calcium. Furthermore, all manifestations of contractility produced by indirect stimulation become decreased by sodium and increased by calcium. Again, the cardiac muscle reacts to sodium and calcium in many respects in a different manner than skeletal muscles. Finally, the same kind of contractile tissue, even in two closely related varieties of animals, may react to sodium and calcium in reverse directions.

From the foregoing it is evident that the relations of sodium and calcium to irritability cannot yet be expressed in general terms. While it is undoubtedly desirable here, as elsewhere in Natural Sciences, to uncover general laws which govern the relations of the ions under discussion to the phenomena of irritability of contractile tissues, we should not undertake to formulate such laws on the basis of comparatively scant observations. What we need, therefore, is more extensive studies of the details of these relations.

Before we enter upon the description of our experimental work we wish to call attention to the following points. In the first place, we wish to refer to the fact that, in practically all the numerous studies on the action of calcium upon muscle tissue, calcium was employed in very dilute solutions and nearly always in conjunction with another salt, especially with a sodium salt, either the two together or the calcium salt following the application of a sodium salt. *Calcium alone has rarely been applied.*

We wish further to mention the fact that in the majority of the studies on the action of sodium and calcium upon the irritability of muscle tissue the muscles were *bathed* in the solutions. In only a small number of investigations the solutions were applied by *perfusion* through the arteries. But there is sufficient reason for the belief that the two methods are liable to give divergent results in some respects. In the infusion method, the solutions reach the sarcolemma of each individual muscle fibre through the capillaries, while in the immersion experiments the solution has to pass by diffusion (and osmosis?) through the epimysium, perimysium, and endomysium before it can come in contact with the sarcolemma. Abel[10] states that the cross section of a frog's gastrocnemius which has been immersed for two hours in an acidulated isotonic saline solution and frozen, "shows an outer layer more homogeneous, coagulated, and impregnated with acid and water, while the core of the muscle is normal in appearance." Even after two hours' immersion the solution did not reach the core. It is possible that even within the outer layer a difference might have been found between the muscle fibres in the centre and those at the periphery of each muscle bundle. While immersion may be considered as the normal method for the study of the action of solutions upon the swimming movements of hydromedusæ or upon the various ciliary movements and even upon the movements of the frog's heart, which normally receives its nutrient fluid by direct bathing of its muscle fibres, for skeletal muscles, vascular infusion of the solution to be tested must be considered as the more normal method of investigation.

EXPERIMENTAL CONTRIBUTIONS.

Methods. — The experiments were made at various times between October and July on the nerves and muscles of Rana pipiens and Rana clamitans which were obtained from various regions in the country. Some of the frogs remained in the cool tank of the Institute for months. Sodium and calcium were employed exclusively as chlorides in pure $m/10$ solutions. Except in one series of experiments, to be mentioned later, the solutions were brought to the tissues by intravascular infusion through the abdominal aorta or through an iliac artery. The method we employed was nearly the same as was employed and described some years ago by Harvey Cushing.[11]

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[10] ABEL: Journal of biological chemistry, 1907, iii; Proceedings of the American Society of Biological Chemistry, viii.

[11] HARVEY CUSHING: This journal, 1901, vi, p. 77. LOCKE (Centralblatt für Physiologie, 1902, xv, p. 537) reproaches Cushing for not giving CARSLAW credit for the method of infusion. Carslaw used that method in LUDWIG's Laboratory (Archiv für Physiologie, 1887). Cushing, however, carried out his investigations in Berne in the laboratory of Professor KRONECKER, who described this method sixteen years previous to Carslaw (Arbeiten aus der physiologischen Anstalt zu Leipzig, 1871, p. 690).

A frog, the brain and cord of which had been previously destroyed was fastened on a board with the dorsum upward. The removal of the os coccyx exposed the abdominal aorta and the lumbar plexus. A suitable glass cannula was introduced into the peripheral end of the aorta and connected by means of a suitable arrangement with two or three graduated burettes which contained the solutions to be tested. One iliac artery was clamped off. By cutting the abdominal vein or by removing the entire abdominal viscera, the returning infused solution was given a free escape. The tendon of one of the gastrocnemius muscles was isolated, separated from its attachment and connected with a lever which marked the contractions upon the drum of a kymograph.

For indirect stimulation the corresponding nerve plexus was placed in a well-secured electrode connected with a Petzold induction coil of a Kronecker pattern. For direct stimulation needle electrodes were inserted at both ends of the belly of the muscle. In most instances separate coils were used for direct and indirect stimulations. The induction coils were armed with one Daniell cell in the primary circuit. Each stimulus consisted of a make and a break shock, which followed each other so quickly as to produce a single contraction. It was produced by means of a metronome interpolated in the primary circuit. In a number of experiments an instantaneous tetanizing current was used, that is, the automatic hammer in the primary circuit was allowed to go continuously, and at stated intervals the secondary circuit was closed automatically for an instant by the metronome contact. In a small number of experiments the stimulations were repeated every four seconds, for the purpose of obtaining a fatigue curve. In the great majority of cases, however, stimulation was done about once in a minute. Maximal stimuli were used, the necessary strength of which was established before starting the infusion.

THE EFFECTS OF SODIUM CHLORID.

Indirect irritability. — Carslaw [12] noticed that after prolonged irrigation of the muscles of a frog's leg with 0.5 per cent or 0.7 per cent sodium chlorid the contractions produced by stimulation of the

[12] CARSLAW: Archiv für Anatomie und Physiologie, 1887, p. 492.

lumbar plexus with single induction shocks became greatly diminished: the effects of tetanizing stimulations were less affected. Locke[13] found that after bathing sartorius muscles of temporaria in 0.6 per cent NaCl for fifteen or twenty minutes, stimulation of the nerve with single induction shocks produced no effect upon the muscle, while tetanizing stimuli were still effective. After one or two hours bathing of the muscle, indirect irritability disappeared completely. H. Cushing[14] stimulated the lumbar plexus of frogs every four seconds with single break shocks, and found that after continuous irrigation of the leg muscles of the frog with 0.6 per cent sodium chlorid fatigue set in very early for the indirect irritability, while the direct irritability persisted for at least seventy-two hours. In the studies of Kronecker and Poliakoff[15] it was established that after irrigating the legs of frogs with 0.7 per cent NaCl for one hour, stimulation of the lumbar plexus becomes ineffective even for maximum stimuli, while the direct irritability of the muscles still persists. In other words, these authors have established the fact that "physiological" solutions of sodium chlorid exert sooner or later a "curare-like" action upon the nerve-muscle preparation of the frog. That the effect was indeed due to a paralysis of the nerve endings and not to that of the nerve fibres was shown by Locke, who found that prolonged bathing of nerve trunks in these saline solutions does not affect their irritability.

All these observations were made on European frogs. Even the observations of Cushing, which were published in this country, were based upon experiments carried out at the Hallerianum in Berne. As far as we know, no such experiments were made on our American frogs. We shall therefore report the results of our observations on the action of sodium chlorid upon the indirect irritability.

In a few experiments the action of sodium chlorid was studied, as in the experiments of Cushing, by its influence upon the development of fatigue of the nerve endings. In these experiments the lumbar plexus was stimulated with double induction shocks as described above, every four seconds, and with a strength of current which gave originally a maximum contraction. The indirect irritability disappeared in all cases with a rapidity which was in proportion to the rapidity of infusion of the saline solution. For instance, the indirect irritability disappeared within two minutes when the rate of infusion was 7½ c.c. per minute; or in four minutes when the rate was 5½ c.c. per minute, while, on the other hand, in one experiment, in which a rate of about 3 c.c. per minute was employed, it took ten minutes to abolish the indirect irritability. However, we soon discarded this method, because we found that even without any perfusion the contractions were often abolished in less than twenty minutes, simply by fatigue alone. In the subsequent experiments the nerve was stimulated not oftener than once in thirty seconds and in most cases not even as often as that.

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[13] Locke: Centralblatt für Physiologie, 1902, xv, p. 537.

[14] Cushing: *Loc. cit.*

[15] Poliakoff: Zeitschrift für Biologie, 1903, xl, p. 23.

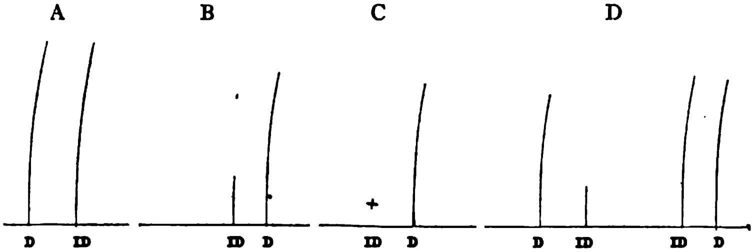

FIGURE 1. — Frog 61, Feb. 2. Primary infusion with $m/10$ NaCl and secondary infusion with $m/10$ $CaCl_2$. *D* = direct, *ID* = indirect. + = no response to stimulation. Maximal contractions produced by short tetanizing current. Both stimulations with the same apparatus; direct, with 100 mm., indirect, with 220 mm. coil distance. *A*, *B*, *C*, and *D* represent sections from the tracing at various stages of the experiment. *A* represents maximal contractions before starting the infusion; *B*, the contractions after 10 c.c.; C, the response after 22 c.c. of NaCl were in. Indirect abolished. Time of infusion, twenty-eight minutes. *D* presents the returning of the indirect irritability soon after the infusion of $m/10$ $CaCl_2$ was started. 3 drops of $CaCl_2$ ran in.

Our results agree in general with those obtained from experiments made on European frogs. The indirect irritability disappeared completely sooner or later, while the direct irritability still persisted. (See *C* in Fig. 1, and *B* in Figs. 2 and 3.) There appeared to be, however, an individual variation regarding the rapidity with which the indirect irritability was affected. The longest interval between the beginning of the irrigation and the complete disappearance of the irritability amounted to sixty-six minutes, during which time 69 c.c. of the saline passed through the muscle; the shortest interval amounted to only eight and one-half minutes with an infusion of but 8 c.c. of the saline. Low temperature seemed to favor the abolition of the irritability. For nine frogs, kept during February in the cool tank without special precautions, the average duration of the interval amounted to forty-four minutes with an average amount of 37 c.c. of the solution. For nine other frogs, kept in the refrigerator at a temperature only two or three degrees above the freezing point, the average duration of the interval amounted to twenty-two minutes with an average amount of 19 c.c. of the perfused solution. However, in the last-mentioned frogs there was quite a striking difference between six of the animals on one hand and three on the other hand. The average duration of the interval for these six frogs was twelve minutes, with an average amount of 12 c.c. of saline; while for the three remaining frogs the average interval amounted to forty-one minutes with an average of 33 c.c. of the saline. In other words, three of the nine frogs kept in the refrigerator behaved nearly the same as the frogs from the tank.

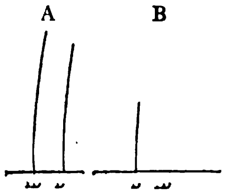

FIGURE 2. — Frog 109, March 30. Method same as in Fig. 1. Direct was stimulated with 60 mm. coil distance on a Kronecker and indirect stimulated with 100 mm. coil distance on a Petzold apparatus. B presents the responses after an infusion of 41 c.c. of $m/10$ NaCl in thirty-four minutes. Indirect abolished. Direct reduced to about one half of the original size.

Direct irritability. — In the foregoing we stated that in all of our experiments the indirect irritability was abolished, while the direct irritability still persisted. This does not mean that the direct irritability of the gastrocnemius muscle suffered no reduction by the irrigation with the sodium chlorid solution. In practically all cases, at the time when the indirect irritability was completely abolished, direct stimulation of the muscle with the original maximum strength of stimulus brought on a contraction which was lower than the original response. In most instances the reduction in the height of the contraction amounted to about one third of the original size, and in a few instances the contraction was little more than one half of the original height. (See *B* in Fig. 2.) Milder temperatures seem to favor the reduction of the direct irritability. Similar results can be found noted also in the protocols of the experiments published by Poliakoff.

RECOVERY OF THE IRRITABILITY BY CALCIUM CHLORID.

Recovery of the indirect irritability. — Locke[16] discovered that the indirect irritability, which was abolished by bathing the sartorius muscle in a pure solution of 0.6 per cent sodium chlorid could be restored in three to five minutes by the addition to the solution of 0.02 per cent calcium chlorid. Cushing[17] and Poliakoff[18] confirmed this observation; the lost indirect irritability was restored, in the experiments of the last-named authors, by a subsequent perfusion of the frog's leg with a Ringer solution.

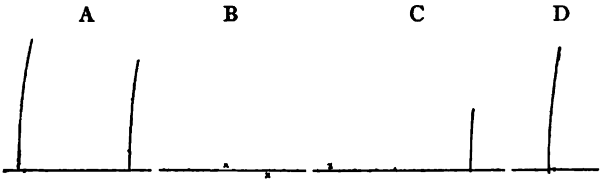

FIGURE 3. — Frog 43, Feb. 1. Primary infusion of $m/10$ $CaCl_2$ and secondary infusion of $m/10$ NaCl. Lumbar plexus alone (indirect) stimulated by double shocks. Maximal contractions. *A* shows contractions before and after starting the infusion of calcium chlorid. *B* shows complete abolition of indirect irritability after 3 c.c. $CaCl_2$. *C* and *D* show return of contractions after starting infusion of NaCl. 4½ c.c. ran in.

In our experiments *we employed pure m/10 solutions of calcium chlorid.* As far as we know, such an attempt was never made before. There was no failure, and the results were striking. In nearly all cases the subsequent infusion of less than 1 c.c. of the calcium solution was sufficient to restore the lost indirect irritability; in some instances the recovery followed after an infusion of only 0.3 c.c. and sometimes even less than that. (See *D* in Fig. 1.) The recovery was prompt even in experiments in which much sodium chlorid was perfused before a complete abolition of the indirect irritability was brought about, or even when the infusion of sodium chlorid continued for some time after the disappearance of the indirect irritability. A perceptible response, on stimulation of the lumbar plexus, could be noticed in practically all cases immediately after starting the infusion of the calcium chlorid solution. As to the degree of recovery in some experiments it was complete, the same maximum stimulus producing a contraction of the same size as the one produced before any infusion took place. In other experiments the recovery was less complete, on account of the depressing action of the calcium chlorid, which sets in very soon, and which we shall discuss presently.

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[16] LOCKE: *Loc. cit.* [17] CUSHING: *Loc. cit.* [18] POLIAKOFF: *Loc. cit.*

The recovery of the direct irritability. — We have stated before that in our experiments in most instances the infusion of sodium chlorid caused a moderate reduction of the direct irritability of the muscle. We may add now that in such experiments the subsequent infusion of *m*/10 calcium solution apparently affected favorably the reduction of this irritability of the muscle. However, moderate as this loss was, its recovery was less evident and less prompt than the recovery of the completely lost indirect irritability of the muscle.

THE PRIMARY ACTION OF CALCIUM CHLORID.

The primary effect of a pure *m*/10 solution of calcium chlorid upon the direct and indirect excitability of muscle has been studied very little. Loeb[19] and Overton[20] mention briefly the destructive effect of such a solution upon the direct irritability; they used the bathing method. No studies at all were made, at least as far as we know, upon the primary action which calcium in *m*/10 solution may exert upon the indirect irritability; or upon direct irritability when the muscle is irrigated with this solution. Nor do we know of any studies upon the recovery from primary depressing effects of the calcium solution by means of the infusion of sodium chlorid.

The abolition of the indirect irritability by calcium. — In one series of experiments we studied the influence of the intravascular infusion of a pure *m*/10 solution of calcium chlorid upon the indirect excitability of the gastrocnemius muscle. In these cases the electric stimulus was applied to the lumbar plexus which was carefully protected from any contact with the calcium solution. In a few experiments the plexus was stimulated every four seconds. In ten experiments the stimulation was repeated only after long intervals — to avoid the factor of fatigue. In these experiments the infusion was administered through the corresponding iliac artery. The stimulation consisted, as described before, of double shocks obtained with the aid of a metronome from a Kronecker or a Petzold induction coil. In all cases maximal stimuli were used. In some experiments after the reaction was completely abolished for these stimuli the effect of much stronger stimuli was also tested. The action of the calcium in the fatigue experiments did not differ from the effects obtained in the experiments in which the factor of fatigue was avoided. We shall therefore confine our report to the results derived from the latter experiments.

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[19] LOEB: Fick's Festschrift, Braunschweig, 1899, p. 101.

[20] OVERTON: Archiv, 1904, cv, p. 176.

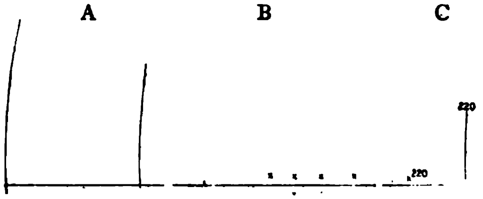

FIGURE 4. — Frog 49, Feb. 4. Method same as Fig. 3. Original stimulus 220 mm. coil distance on a Kronecker apparatus. *A* shows contractions before and after starting infusion of $CaCl_2$. *B*, after 6 c.c. of $CaCl_2$ were infused; no response, even to 50 mm. coil distance. *C* shows a fair response to 220 mm. coil distance again after infusion of only 2 c.c, of NaCl.

In every experiment the calcium solution abolished the indirect irritability. After the infusion of a comparatively small quantity of the solution, stimulation with the original strength of stimulus produced no effect whatsoever (see Fig. 3, *B*), and, in the experiments in which this was tested, stronger stimuli also failed to produce any effect. (See Fig. 4, *B*.) The smallest quantity which caused such a complete depression was 2.5 c.c., which passed through the muscles in six and one half minutes. The largest quantity amounted to 9 c.c., which ran through the muscle in eleven minutes. The following figures present the average for the ten experiments: 5.3 c.c. of the solution passed through the muscles in seven and one half minutes. In most of the experiments the onset of the reduction developed slowly, while the further depressing action progressed quite rapidly, the line connecting the tops of the contractions presenting at first a convex and later a concave curve. In a few instances, however, this curve formed a straight line, the rate of loss of the irritability being uniform from start to finish. This occurred in the experiments in which it took a larger quantity of the solution and a longer time to run in before the irritability was completely inhibited.

Recovery from the calcium depression. — In some experiments the infusion of the calcium solution was stopped as soon as complete loss of irritability was reached. If the muscles were then left to themselves, no spontaneous recovery ever took place. Neither did the simple washing out seem to have any restoring effect. In some instances distilled water was infused through the muscles without causing any change. It was found, nevertheless, that this complete abolition of the excitability by a *m*/10 calcium solution is readily reversible, and the agent which causes the recovery is sodium chlorid also in *m*/10 solution, that is, the restoration of the irritability is accomplished by an agent which, when applied primarily, causes its abolition. (See Fig. 3, *C* and *D*, and Fig. 4, *C*). In practically all instances the subsequent infusion of a *m*/10 solution of sodium chlorid caused a return of the lost irritability, and in most cases it was restored to its original degree. The first appearance of a contraction takes place soon after only 1 c.c. of the sodium solution runs in. The rapidity of the recovery and its completeness depends largely upon the amount of calcium which passed through the muscle in the preceding infusion. In many experiments calcium was permitted to continue to flow through the muscle for some time after all irritability was gone. Under these circumstances the more of the calcium that ran through the muscle the slower the recovery of the contractility by the sodium. In one case 30 c.c. of the calcium solution were permitted to perfuse the muscle; in this solitary case no recovery of the indirect irritability of the muscle by sodium took place. In some experiments the sodium was permitted to continue to flow long after the recovery took place. In these cases the contractions began to go down again, apparently through the action of the sodium.

In most of the experiments after the recovery by sodium a second injection of the calcium solution was tried. In these cases the calcium again brought down the irritability to zero. But in the second injections a larger quantity was required to abolish the excitability and the effect developed much slower than in the first infusion. A subsequent injection of sodium chlorid restored again the lost irritability. Although the second recovery by sodium developed somewhat slower than in the first infusion, it was more prompt in its action than the second infusion of the calcium solution.

These experiments have demonstrated, then, that a vascular infusion into the muscle of a pure solution of calcium chlorid, isotonic with 0.6 per cent of sodium chlorid, is capable of depressing the irritability so completely that excitations of the motor nerve with maximal electrical stimuli fail to elicit any response. They demonstrated further that this complete depression of the irritability is promptly reversible;

a subsequent infusion of a pure *m*/10 solution of sodium chlorid restores the lost irritability fairly rapidly. The action of calcium would seem therefore not to differ in principle from that of sodium. Sodium chlorid in its primary action depresses the excitability of the motor nerve, and this depression is restored by calcium; calcium also, in its primary action, depresses the excitability of the nerve, and this depression is restored by sodium. However, for sodium chlorid it was shown that the depression caused by it affects essentially the nerve endings, whereas the direct irritability of the muscle suffers comparatively little from the influence of this solution. In other words, sodium chlorid affects greatly the indirect and but little the direct irritability. Now what is the nature of the depressing action of calcium; does it affect also essentially the nerve endings, or is the depression due to an abolition of the direct irritability of the muscle? The few facts contained in the literature with reference to the action of pure solutions of calcium chlorid throw no light upon this question. They deal exclusively with the complete destruction of the direct irritability by prolonged bathing of the muscle in the calcium solution.

The action of calcium chlorid upon direct and indirect irritability studied simultaneously. — In the foregoing series of experiments we met with an experiment in which, as already mentioned, after an infusion of 30 c.c. of the calcium solution the lost irritability of the motor nerve could not be recovered any more. In this experiment we found that a direct stimulation still caused a definite contraction of the muscle. It seemed, in this case at least, that the entire loss of indirect irritability could not have been caused by the loss of the muscle contractility. Our next step was therefore to study direct and indirect irritability simultaneously throughout the experiment. For testing of the direct irritability two needle electrodes were inserted into the body of the muscle, one electrode near each pole. In some cases both direct and indirect stimulations were accomplished by the same induction apparatus with the aid of a pole commutator; in most cases, however, nerve and muscle were stimulated by separate induction apparatuses. We may remark here in parenthesis that when both kinds of stimulations were accomplished by one and the same coil it was invariably found that to obtain a maximum contraction a much stronger stimulus was required for the muscle than for the nerve, or, in other words, the same strength of stimulus elicited from the nerve a much stronger contraction than from the muscle — a well-known fact to which we shall refer again later. (See *A* in all figures.)

For some external reasons these various experiments were carried out with some interruptions. A series of six experiments were performed during the months of January and February. (In these experiments the stimulations were executed not by single make and break shocks but by a short tetanizing current, the metronome being interpolated in the secondary circuit.) These experiments gave us a surprise. In all six experiments the infusion of the calcium solution brought down the indirect and the direct irritability in a nearly parallel manner, and the subsequent infusion of sodium chlorid gave a similar parallel recovery of both forms of irritability (*A*, *B*, and *C* in Fig. 5). The average amount of the solution of calcium chlorid which sufficed to completely abolish both forms of irritability was 5.6 c.c., which ran through the muscle in an average time of 10.9 minutes. These figures differ little from those obtained in the experiments on the indirect irritability alone. The new series of experiments seemed to indicate that there is a very close connection between the loss of both forms of excitability, and that there was, therefore, no compelling reason for an assumption that calcium chlorid acts, like sodium chlorid, especially upon the motor nerve endings; in other words, the possibility was not excluded that calcium depresses the contractile substance, and the loss of the indirect irritability was only secondary to this depression.

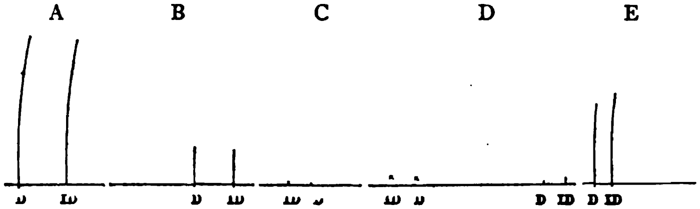

FIGURE 5. — Frog 58, Feb. 9. Primary infusion of *m*/10 $CaCl_2$ and secondary infusion of *m*/10 NaCl. Maximal contractions. Stimulation with double shocks; lumbar plexus (indirect) with 300 mm. and muscle (direct) with 120 mm. coil distance. *A*, before starting infusion of $CaCl_2$, *B*, after infusion of 5 c.c., *C*, after infusion of 8 1/2 c.c., *D*, complete inhibition at the end of the calcium infusion and the reappearance after starting the infusion of NaCl of both *D* and *ID*. *E*, after 4 c.c. NaCl. Direct and indirect irritability went down and recovered parallel.

The next series of experiments was begun on the 18th of March. (In these experiments the metronome was in the primary circuit, and each stimulus consisted of a make and break shock as described above.) In the first experiment a large green bull frog was used. The result was again a complete surprise. As in previous experiments the indirect irritability became rapidly affected; after eight minutes, when 4½ c.c. of the solution had passed through the muscle, stimulation of the nerve elicited no response. The direct irritability, however, showed at that time not a sign of reduction; and even after 18 c.c. of the solution had run through the muscle, direct irritability was unaffected. (See Fig. 6 and Fig. 7.) This series of experiments was continued through the remainder of March and the first part of April. We met with no other instance in which a continuation of the calcium infusion would not finally abolish or at least greatly reduce also the direct irritability of the muscle. In practically none of this series of experiments, however, was there a parallel loss of direct and indirect irritability such as was obtained in the first six experiments performed in February. When the infusion reached the stage in which the stimulation of the nerve no longer produced a response, direct stimulation of the muscle would still give a contraction which was either not much smaller than the original size, or would vary in height between two thirds and one third of the original contraction. At any rate, in these later experiments, both forms of irritability never came down at the same time.

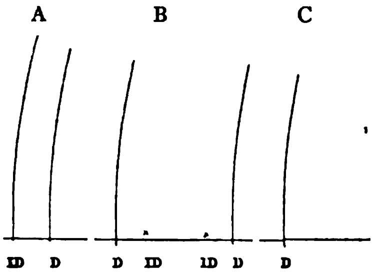

FIGURE 6. — Frog 95, March 18. Method same as in Fig. 5. Primary infusion of $m/10$ $CaCl_2$ only. *A*, before starting infusion. *B*, after infusion of 5 c.c. Indirect completely abolished. Direct persists. *C*, after 18 c.c. $CaCl_2$. Direct still persists.

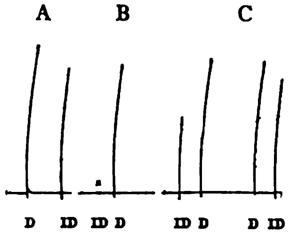

FIGURE 7. — Frog 106, March 26. Method same as in Fig. 5. Inhibition by primary infusion of $CaCl_2$ and recovery by secondary infusion of NaCl. *A*, contractions before starting infusion of $CaCl_2$. *B* shows inhibition of indirect and persistence of direct irritability after infusion of 8 1/2 c.c. of $CaCl_2$. *C*, recovery of indirect after infusion of 1 c.c. NaCl.

In looking for a cause for this discrepancy between the results of the two series of experiments, our attention was directed, in the first place, to the difference in the temperature as a possible factor. The first series of experiments, in which there was a manifest parallelism between the two forms of irritability, were carried out in the early part of February, while the other experiments were made practically at the beginning of spring. Ringer stated that "cold favors the action of calcium," and "calcium chlorid exerts its influence better in midwinter than in the later part of the winter." However, the influence of the calcium in Ringer's experiments referred to its neutralizing action upon the depressing effect of sodium chlorid, and cold therefore seemed to be a favorable factor in the direction of sustaining the irritability of the muscle. In our experiments the action of the temperature seemed to be in the reverse direction: calcium seemed to reduce the direct irritability better at a lower than at a higher temperature. On the other hand there is this difference between our studies and those of Ringer. Ringer dealt in his experiments with the secondary effect of calcium, while in our experiments the primary action of calcium was studied. We decided therefore to test the influence of the temperature in our line of work by direct experimentation. In some of these experiments the perfused leg was cooled during the entire experiment by the direct application of cold saline or crushed ice. In many other experiments the animals were kept for various lengths of time, previous to the experiment, in the refrigerator at a temperature only a few degrees above the freezing point; in some experiments the animals remained in the refrigerator from April to June. The outcome of these experiments was definite; there was a striking difference between the cooled and non-cooled animals. While in the non-cooled animals the resistance of the direct irritability to the action of calcium was in practically every case greater than that

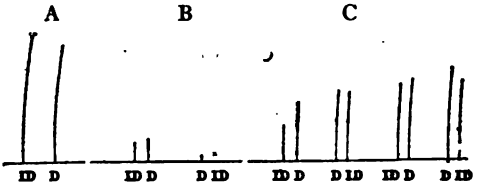

FIGURE 8. — Frog 111, March 31. In ice bath during experiment. Method same as in Fig. 7. *A* shows contractions before starting infusion of $CaCl_2$. *B*, after infusion of 2 c.c. $CaCl_2$. *C*, the infusion of 2 c.c. NaCl. Direct and indirect irritability came down and recovered practically parallel.

of the indirect (in some instances there was hardly any reduction in the direct at the time when the indirect was completely abolished (see Fig. 6 and Fig. 7), in all of the cooled animals there was only a moderate, very little, or no difference at all between the two kinds of irritability (see Fig. 8). The most striking influence of the temperature was in the experiments carried out during the month of June; at this time in all of the non-cooled animals, the direct irritability showed a great resistance to the depressing effect of the calcium chlorid (see Figs. 9 and 10).

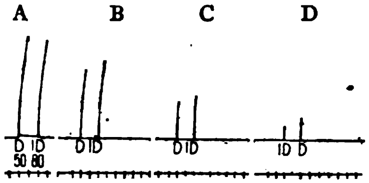

FIGURE 9. — Frog 202, June 18. Kept in refrigerator from April 1 to June 18. Method same as in Fig. 7. Primary action of $CaCl_2$ only. Direct and indirect irritability came down parallel. 10 c.c. infused.

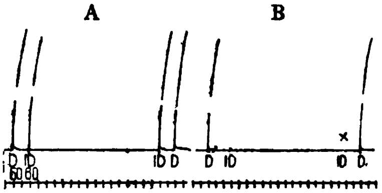

FIGURE 10. — Frog 204, June 18. Frog at room temperature. Method same as in Fig. 7. Only primary action of $CaCl_2$. Indirect irritability inhibited while direct persists. 2½ c.c. infused.

Besides the temperature (and perhaps also the season), there are probably still other conditions which are capable of modifying the depressing action of the calcium solution upon the direct irritability and which in one way or another influenced our results. There were differences in the degree of response to the action of the calcium solution in the various experiments which were carried out apparently under similar conditions of temperature. We shall not attempt to analyze the possible nature of these conditions. For the present we may adopt the dictum of Ringer that the degree of irritability depends in a measure upon the "individual variations" of the animals.

The indirect irritability was not strikingly influenced by cold. For thirteen "cold frogs" the average quantity of the calcium chlorid solution which caused complete abolition of the indirect irritability amounted to 5.4 c.c. In the six animals experimented upon in February in which both kinds of irritability disappeared together, the average quantity of the solution amounted to 5.6 c.c. About the same average amount was necessary for abolition of the indirect irritability in the non-cooled animals experimented upon in the spring. However, we have to record that among the "cold frogs" there were two animals which responded only to exceptionally large quantities of the solution. The direct irritability was abolished in one frog when 22 c.c. passed through in thirty-three minutes, and in the other frog when 21 c.c. of the calcium solution passed through in twenty-two minutes. These exceptionally large figures were not included in the averages derived from the thirteen other frogs.

Recovery of calcium depression by sodium in the simultaneous studies.— In most of the experiments in which the primary action of calcium chlorid was studied in its depressing effect upon both kinds of irritability, the secondary action of sodium was also studied upon both kinds of irritability. We stated above, when reporting the experiments in which the nerve plexus alone was stimulated, that the lost indirect irritability was restored by a subsequent infusion of sodium. This also holds good in general for the direct irritability. In the experiments performed at a milder temperature at which the direct irritability was less depressed than that of the indirect, the recovery of the direct irritability by the infusion of sodium was more rapid than that of the indirect. In the experiments in which the loss of both kinds of irritability occurred more or less parallel the recovery took place in general in about the same relation (see Figs. 7 to 10). We have, however, to record one exception; there was one experiment in which a complete recovery of the indirect took place, while there was practically no recovery at all of the direct irritability, although in this experiment the loss of the irritabilities occurred exactly parallel. Otherwise the recovery of the direct irritability was generally slightly ahead of the indirect. This was especially evident when a good deal of calcium was permitted to pass through the muscle. Under these circumstances the indirect would sometimes not recover at all, while some recovery of the direct irritability would still occur. Furthermore, while there might have been no difference in the final degree of recovery, it was often evident that during the development of the recovery the direct irritability returned faster than the indirect.

When a good deal of sodium was used for the restoration of the irritabilities it often happened that the indirect irritability began to fail again. This, however, was simply due to the "curare-like" action of sodium. In these cases the infusion of a small quantity of calcium chloride would again rapidly restore the lost indirect irritability— which brings out strikingly the contrast between the primary and the secondary action of calcium.

Our experiments with the stimulation of the lumbar plexus alone gave rise, as stated above, to the question whether the depressing action of calcium, noticed in these experiments, was due to a primary depression of the indirect irritability, or was only secondary to the abolition of the direct irritability of the muscle. This question we may now answer with the statement that calcium in its primary action is capable of depressing both direct and indirect irritability independently of one another, and that both depressions may run a different course. It was observed, in the first place, that in spring frogs, and at higher temperature in general, the direct irritability manifests a much greater resistance to the depressing action of calcium than the indirect. Furthermore, the recovery from the calcium depression through the influence of sodium shows also a somewhat different course for both forms of irritability. In one experiment sodium chlorid was capable of restoring only the indirect and not the direct irritability. On the other hand, in all other instances the recovery of the direct irritability ran ahead of that of the indirect irritability.

The action of calcium upon nerve fibres. — It remained yet to be investigated whether, in the depressing action of calcium upon the indirect irritability, the nerve fibres or the nerve endings were affected, in other words, whether calcium, like sodium, exerted a curare-like action. We studied therefore the action of *m*/10 calcium chlorid upon nerve trunks. For these experiments we had to take recourse to the bathing method, which was carried out as follows.[21] A loop of the sciatic nerve was kept down in a very small glass cup (made from a short piece of glass tubing) by means of small plugs of absorbent cotton. The cup was filled with a *m*/10 solution of calcium chlorid, and the plugs of cotton were frequently saturated with this solution. The plexus above the cup and the part of the nerve between the cup and muscle were covered with pledgets of cotton saturated with Ringer's solution. Tracings were obtained from the gastrocnemius muscle in the manner described above. The skin of the leg was not removed.

In these experiments the actions of calcium chlorid and of sodium chlorid were tested at the same time on the same frog, using one leg for calcium and the other for sodium. In either case only the conductivity of the nerve trunks was tested; that is, the nerve was stimulated only above the loop, and not the loop itself, the latter being left undisturbed in the cup. The lumbar plexus was tested at various intervals with a constant, maximum strength of stimulus; when the response began to fail, also the part of the nerve near the muscle was tested. The results, briefly stated, were as follows: in nine out of ten experiments the calcium nerve lost its conductivity about two or three hours after bathing began. After complete loss of conductivity the change of the nerve into a sodium chlorid bath restored the conductivity completely. The restoration was the more rapid the sooner the conductivity was abolished by the calcium bath. The sodium chlorid bath caused no loss of conductivity in the sciatic nerve; after many hours, stimulation of the nerve between the bath and the muscle showed the same reduction in the reaction as the portion of nerve beyond the bath.

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[21] See Proceedings of Society for Experimental Biology and Medicine, 1908–1909, vi, p. 104.

According to these experiments calcium chlorid in *m*/10 solution abolished the conductivity in nerve only after hours of bathing. In our perfusion experiments the response to the stimulation of the lumbar plexus was abolished in a few minutes; this abolition, therefore, could have been due only to the action of the calcium chlorid upon the nerve endings and not to its action upon the conductivity of the nerve fibres.

DISCUSSION.

Let us now pass in review the results obtained in these investigations. The experiments on the primary action of sodium and the secondary actions of calcium established, in the first place, that also in Rana pipiens and Rana clamitans, irrigations with sodium chlorid will abolish the indirect irritability and subsequent irrigation with calcium chlorid will restore it. These experiments brought out, furthermore, some details which deserve to be especially mentioned. In the experiments of Locke[22] in which the sartorius muscles of Rana temporaria were subjected to a bath of 0.7 per cent sodium chlorid, the indirect irritability disappeared in fifteen minutes for stimulation with single shocks, and in one or two hours for tetanizing stimuli. For the gastrocnemius muscle, however, Overton[23] states that at room temperature it may stay in the *bath twenty to forty hours* before the indirect irritability disappears and at low temperature it may persist even as long as *fifty hours*. In our experiments in which the sodium chlorid was applied by infusion the indirect irritability of the gastrocnemius disappeared in some instances in *less than nine minutes*. The longest interval was only a little over one hour. That shows *how much more readily and intimately the solution is brought to the muscle fibre by infusion than by bathing*. Our results agree with those of Poliakoff, who found also that the indirect irritability of the gastrocnemius disappeared after irrigation for one hour with sodium chlorid. The conclusions of Poliakoff contain, however, the statement that the indirect irritability disappears *suddenly*. In our experiments the disappearance was rather a *gradual one*.

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[22] LOCKE: *Loc. cit.*

[23] OVERTON: *Loc. cit.*, p. 264.

The restoration of the indirect irritability was accomplished in our experiments by pure calcium chlorid. In the experiments of other investigators either Ringer's solution or a solution of sodium chlorid plus calcium chlorid was employed for the restoration. The recovery in our experiments was very rapid, even when the muscle was previously irrigated by a good deal of sodium. In Locke's experiments on the sartorius muscle the recovery took place also very rapidly, — "in three to five minutes." There are no statements in the literature as to the recovery of the gastrocnemius muscle after the abolition by prolonged bathing. (In Poliakoff's paper there are protocols of experiments in which the recovery of the gastrocnemius took place after an irrigation with Ringer's solution for forty-five minutes.)

We found further that irrigation with sodium chlorid reduces also direct irritability, a fact which is in agreement with an expressed statement of Poliakoff. The reducing effect, however, is a very moderate one, especially in comparison with the effect upon the indirect irritability. Langley[24] stated recently that he cannot find "that the difference in the time of the two events (loss of indirect and direct irritability) is so great as is described by some observers." In our experiments the difference was invariably considerable.

We should mention that in our experiments subsequent irrigation with calcium chlorid exerted a restoring influence also upon the direct irritability, but the recovery was less marked than that of the indirect irritability. As far as we know, nobody mentions this fact directly; but it is contained in the various protocols published by Poliakoff.

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[24] LANGLEY: Journal of physiology, 1911, xl; Proceedings of the Physiological Society, p. xxiv.

Our experiments brought out also the noteworthy circumstance that lower temperature seems to favor the depressing action of sodium upon the indirect irritability; that is, in cooled frogs the indirect irritability seemed to disappear after a shorter irrigation than in non-cooled animals. It is not probable that this was due to an additional depressing influence of the cold upon the motor nerve endings, for these nerve structures seem to survive rather longer in lower than in higher temperatures.

We may finally say that our observations confirm in general the statement of Locke that sodium chlorid does not lower the irritability of nerve fibres, and that therefore the observed abolition of the indirect irritability could be due only to the depressing action of sodium chlorid upon the motor nerve ending.

Regarding the primary action of calcium and the secondary action of sodium our experiments brought to light instructive and new facts. As stated above, the little which is mentioned in the literature on this subject refers to the destructive action of calcium chlorid isosmotic with 0.7 per cent sodium chlorid. Loeb [25] states briefly that such a solution destroys the faradic (direct) irritability of muscles, and Overton's [26] conclusion reads that muscles die in it. (In one of the two protocols published by Overton, contractility disappeared after four and a half hours, and in the other a trace of the contractility was present even after twenty-four hours.) Both investigators studied the action of the calcium solutions by the bath method. With regard to this claim for the primary action of calcium our irrigation experiments do not entitle us to make a definite statement. On the one hand we found not a single instance of an irrevocably destructive effect of $m/10$ calcium chlorid, although in a number of cases the irrigation with calcium was continued for quite a long time after both forms of irritability had disappeared entirely. On the other hand the prolonged irrigations were not pushed far enough and with sufficient repetitions to entitle us to the opposite opinion, namely, that irrigation of muscles with $m/10$ calcium chlorid does not lead to a permanent destruction of both forms of irritability.

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[25] LOEB: Studies in general physiology, Decennial publications, Chicago University, Chicago, 1905, p. 531.

[26] OVERTON: *Loc. cit.*

Our investigations, however, brought out the following new facts: a *primary perfusion of the muscles with m/10 calcium chlorid is capable of completely inhibiting all irritability of nerve and muscle; but this inhibition is promptly reversible, and the reversion is accomplished by a subsequent irrigation with m/10 sodium chlorid.* Calcium chlorid in its primary action abolished, in our experiments, the irritability of nerve trunks, of muscle tissue, and of the motor nerve endings. The abolition of the irritability of the nerve endings by calcium chlorid is of special interest, since, on the one hand, it is calcium, in its secondary action, that restores the irritability of the nerve endings, abolished by sodium, potassium, and magnesium, and, on the other hand, *it puts calcium, with regard to its primary action, in the same line with the other ions which, as just mentioned, exert a curare-like action upon the nerve endings.* Calcium chlorid reduces and even completely abolishes also the direct irritability of muscle tissue. This it does best in the winter season and at an artificially low temperature. On the other hand, change of temperature affects but little the depressing action of calcium upon the motor nerve ending. It was this difference in the behavior towards temperature which enabled us to distinguish between the responses of the motor nerve ending and the muscle tissue to the primary action of calcium. During the spring and the summer the muscle irritability was only tardily affected, while the depression upon the motor nerve endings was as prompt as in the winter season. It may be stated, however, that apparently at all times and in all circumstances *calcium depresses more readily the peripheral nerve endings than the muscle tissue.* Nerve trunks are depressed by calcium more slowly than either the nerve endings or muscle; it took hours to depress the conductivity of the nerve trunks and only minutes to abolish the indirect irritability.

The lost irritability is restored rapidly by sodium chlorid. — In this regard there seems to be little difference between the irritabilities of the three tissues: the conductivity of the nerve trunk returns apparently with the same rapidity as the indirect irritability. The temperature does not seem to be a definite factor in the process of recovery by sodium chlorid.

There are definite differences between the primary actions of calcium and of sodium chlorid upon the various irritabilities. The difference is most striking in the action upon the nerve trunks; the conductivity is abolished by calcium and is practically unaffected by sodium. The difference is also very obvious in the actions upon the direct irritability of the muscle; calcium may depress it completely, while its depression by sodium is only moderate; the depression by both salts is favored by low temperature. The difference in the actions upon the motor nerve endings is only a quantitative one; the abolition of the indirect irritability may be attained by both, but it requires a good deal more of the sodium than of the calcium to accomplish it.

There are very little differences, if any, in the secondary, corrective actions of both ions; sodium chlorid restores the irritability lost by the primary action of calcium salts practically with the same promptness as calcium chlorid restores the irritability lost by sodium chlorid.

This is as far as we intend to go in our statements; that is, we shall stick to our facts.

In view of our new experience with the primary action of calcium and the secondary action of sodium it is obvious that a theory of the nature of the curare-like action of sodium (Overton), or of the nature of irritability (Loeb) developed solely upon the experience with the primary action of sodium and the secondary action of calcium is not likely to be adequate. But we are not ready to replace either of them by other theories. "Any theory constructed from the present facts would be premature and would soon have to be reconstructed. The various hypotheses which one has, and has to have, as a guide to direct the further search for facts, had better not see the light of day before their viability is well assured." (Meltzer and Auer.[27])

Direct muscle irritability and the irritability of the contractile muscle substance. — In connection with the foregoing we wish to make here the following general statement. We discussed above the possibility that the abolition of the indirect irritability by calcium might have been due only to an abolition of the direct irritability, implying hereby as self-evident that in the absence of direct irritability no indirect irritability could be present. This is based upon the assumption that the so-called "direct" irritability of the muscle is identical with the direct irritability of the contractile substance of the muscle.

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[27] MELTZER and AUER: This journal, 1908, xxi, p. 400.

This seems to be indeed in harmony with the tacitly accepted general opinion, although we do not find that this supposition was ever the subject of a special discussion. Now there are some facts and considerations which are capable of raising doubts as to the correctness of this view and to which we wish here to call attention. Of our own investigation we wish to recall here one experiment in which calcium chlorid abolished simultaneously the direct and indirect irritability, and in which, however, *sodium chlorid restored the indirect irritability while the direct irritability remained ineffective.* Now, if the direct stimulation of the muscle is identical with the direct stimulation of the contractile substance and the failure of the direct irritability means failure of the contractile tissue to react to a stimulus, it is difficult to understand how it may happen even in a single case that stimulation of the nerve will produce a contraction, while the contractile tissue in this case is still incapable of responding to a stimulus. Furthermore, there is also a well-established fact in the physiological literature which militates against the identity of the direct stimulation of the muscle with the direct stimulation of the contractile tissue. Since the researches of Remak,[28] Bernard,[29] and especially of Rosenthal,[30] in the fifties of the last century, it has been an acknowledged fact that a much stronger electrical stimulus must be applied to the muscle than to the nerve for the production of contractions of the same degree. In our experiments we had frequent occasions, as described above, to convince ourselves of the correctness of this statement. To obtain a maximal contraction the secondary coil had to be a good deal nearer to the primary when the muscle was stimulated than in the stimulation of the lumbar plexus. The fact is a well-established one and has been seen and recorded by many physiologists of a previous generation. It is surprising that there is no record of it in many of the recent text books.

It seems to us, we ought to bear in mind, that when stimulating the muscle directly, so called, the contractile substance is at all times separated from the stimulus by the layers of connective tissue of the perimysium externum and internum and by the sarcolemma; also by a larger or lesser amount of lymph. That these interfibrillary masses are capable of hindering the transmission of a stimulus, can be learned from the well-known interesting experiments of Kühne[31] and of Biedermann[32] regarding the secondary contraction from muscle to muscle. Normally the conduction of the contraction wave is strictly isolated; the wave is not transmitted from fibre to fibre and certainly not from muscle to muscle. Kühne,[31] however, discovered that the contraction will readily pass from one muscle to another, if the muscles are pressed closely together, whether surface to surface or end to end. Kühne produced in this way a chain of four or five sartorius muscles of frogs which were held together end to end by little presses; all these muscles contracted violently one after another as soon as one of them was stimulated. *The transmission of the contraction, however; failed when fine membranes, for instance, the mesentery or lungs of the frog, were interpolated between two of the muscles.* The contraction of one of these muscles is being brought about by the action current of its contracting fellow muscle, the transmission of which current, however, is impeded by the interpolation of a poor, moist conductor, be it ever so thin. Furthermore, Biedermann[32] found that in dried muscles of the frog circumscribed stimuli bring on a violent tetanus of the entire muscle which wanders over to the neighboring muscle, even if both muscles are only loosely joined together. Kühne thinks that in both cases the absence of the surplus of fluid in these dried or compressed muscles favors the transmission of the current of action from fibre to fibre and from muscle to muscle. It follows from this that the absence of such transmissions in normal muscles is due to the presence of a surplus of lymph within them.

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[28] Remak: Über methodische Elektrisirung gelähmter Muskeln, Berlin, 1856.

[29] Claude Bernard: Comptes rendus, 1857. After Hermann, Handbuch der Physiologie, 1879, i, 1, p. 111.

[30] Rosenthal: Moleschott's Untersuchungen, 1857, iii, p. 185.

We thus see that the presence of lymph within the muscle is an impediment to the transmission of an electric current to the muscle fibre. We saw further that the transmission of the current is prevented by the interpolation of thin tissues such as the mesentery of the frog. We have therefore good reasons for the supposition that a stimulus which is applied "directly" to the muscle, by having to traverse the interfibrillary tissues and fluids, loses a great deal from its original force, and the quantity of the stimulus which finally reaches the contractile substance of the muscle is therefore definitely smaller than that applied to the muscle as a whole. In other words, "direct" stimulation of the muscle is not identical with the stimulation of the contractile substance. Furthermore the resistance of the interfibrillary tissue and fluid to the transmission of the stimulus is surely not of an invariable nature; it may increase or decrease according to various influences. We may therefore say further that the irritability of the muscle is by no means identical with the irritability of the contractile substance.

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[31] KÜHNE: Zeitschrift für Biologie, 1888, xxiv, p. 383; 1890, xxvi, p. 203.

[32] BIEDERMANN: Sitzungsberichte der Akademie der Wissenschaften zu Wien, 1888, xcvii, iii, 7 Juni.

On the other hand, a stimulus which is sent to the muscle through the motor nerve fibres, the neurilemma of which passes over directly into the sarcolemma, reaches the contractile tissue perhaps with greater facility and with less loss of energy. We ought also to remember that the motor nerve is the natural path for the stimulus, and by reaching the muscle through the motor nerve, the stimulus enters the dwelling of the contractile substance through a "door," while in the direct stimulation of the muscle it enters into the dwelling by breaking through its "walls." The terms "direct" and "indirect" stimulation are here misnomers. Entering through a well-constructed path leading to the door is surely more direct than entering through a break made in the wall.

The theory which we offer is then as follows: Stimuli can reach the contractile substance of muscles either through motor nerves or through the complex tissues representing the mass of the muscle. When the stimuli are sent through the nerves, they pass through a natural "door" — the motor nerve endings. When they are applied "directly" to the muscles, the stimuli have to "break through the wall" — that is, through the various layers of connective tissue and interstitial lymph normally surrounding and protecting the contractile substance. Under normal conditions stimuli reach the contractile tissue with greater facility and with less loss of energy through the "door" than through the "wall." Hence the greater readiness in responding to nerve than to muscle stimulation. The irritability of the contractile substance is not identical either with the "direct" or "indirect" irritability. Both the tissue and lymph between the muscle fibres as well as the tissue and lymph of the motor nerve endings, offer resistance to the entrance of the stimulus into the contractile substance, but in different degrees; we assume that normally the motor nerve endings offer less resistance. Various agents change the resistance of the "door" as well as of the "wall," but by no means in a parallel manner. Hence the occurrence of such a result as complete recovery of the "indirect" with no recovery of the "direct" irritability. As to the irritability of the contractile substance itself, we are unable to discuss its nature, or how it is affected by various agents. For the present we do not know of a method of reaching it without involving at the same time the irritability of the tissues constituting its wall or its door.

We offer this theory in the belief that it may prove a fruitful working hypothesis.

SUMMARY.

In the following summary only the most essential points will be presented.

Irrigation of the gastrocnemius with a *m*/10 sodium chlorid solution abolishes completely the irritability of the motor nerve endings in an interval not much longer than one hour and sometimes even in less than ten minutes. Low temperature accelerates this curare-like action. The direct irritability of the muscle is reduced by this irrigation only in a moderate degree. Subsequent irrigation with *m*/10 calcium chlorid restores rapidly both kinds of irritability.

Primary irrigation with *m*/10 calcium chlorid abolishes the indirect irritability much more promptly than the irrigation with sodium chlorid. Low temperature exerts no perceptible influence upon this action. Direct irritability is affected by this irrigation nearly as promptly as the indirect, but only at a low temperature. At the temperature of spring and summer the depression of the direct is far behind that of the indirect irritability, but it is still greater than that caused by the primary action of sodium. Bathing of motor nerve trunks in *m*/10 calcium chlorid abolishes the conductivity in two to three hours.

The lost irritabilities of muscle, nerve endings, and nerve trunks are rapidly restored by irrigation (or bathing) with *m*/10 sodium chlorid.

There is therefore a great similarity between sodium and calcium ions in their primary actions as well as in the secondary: both cause depression in their primary actions, but each one, and calcium more

than sodium, neutralizes in the secondary action the depression caused by the other in the primary action.

Theories of irritability, etc., based solely upon observations of the primary action of sodium and secondary action of calcium, are, in view of the above-mentioned results, necessarily inadequate.

The hypothesis is offered that direct irritability of the muscle is not identical with the irritability of the contractile tissue.

AURICULAR SYSTOLE AND ITS RELATION TO VENTRICULAR OUTPUT.

BY ROBERT A. GESELL.

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THE effects of auricular systole have long been noted. The venous pulse, the arterial pulse, intraventricular pressure, and the apex beat all give evidence of the activity of the auricles. The classical curves of Chauveau and Marey,[1] of intraventricular pressure in the horse, show a small, but well-defined wave before the sharp systolic rise. This same wave was also observed by Fredericq,[2] Hürthle,[3] Rolleston,[4] and others. Von Frey and Krehl[5] were able to record it only on exceptional occasions. Tigerstedt[6] points out that the failure was probably due to the methods of recording, and also to the short A^sV^s interval which might have prevailed. For if ventricular systole follows too closely upon auricular systole, the ventricular wave is invisibly superimposed upon the auricular wave.

The pressure in the auricles, during auricular systole, has been found to mount as high as 22 mm. Hg,[7] and pressure waves within the arteries have been noted to occur, in the horse and man, synchronously with auricular systole. Three suggestions have been made to account for them:

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[1] CHAUVEAU and MAREY: Mémoires de l'Academie de Médecine, 1863, xxvi, p. 296: Reference, TIGERSTEDT, Physiologie des Kreislaufes, 1893, p. 99.

[2] FREDERICQ: Travaux du laboratoire, 1888, ii, pp. 73, 74: Reference, TIGERSTEDT, Physiologie des Kreislaufes, 1893, p. 99.

[3] HÜRTHLE: Archiv für die gesammte Physiologie, 1891, lxiv, p. 55.

[4] ROLLESTON: Journal of physiology, viii, p. 242.

[5] v. FREY and KREHL: Archiv für Anatomie und Physiologie, phys. Abth., 1890, p. 61.

[6] TIGERSTEDT: Physiologie des Kreislaufes, 1893, p. 100.

[7] MAGINI: Archives italiennes de biologie, 1887, viii, p. 127; Reference, TIGERSTEDT, Physiologie des Kreislaufes, 1893, p. 90.

1. Direct transmission of auricular pressure from the ventricles into the aorta.[8]

2. Indirect transmission by beating of the jugular pulse upon the carotid.

3. Mechanical effect of auricular systole upon the root of the aorta.[9]

Erlanger [10] has shown in a case of heart block in man that the waves were undoubtedly present in the arteries. The same author,[11] in his work on heart block in the dog, noticed a distinct bulging of the ventricles with every auricular systole, and was able to record the movements of both the ventricles and auricles, with one suspension from the bulging part of the ventricles.

It is hardly likely that the auricles act merely as reservoirs; it would seem that their contractions must help materially in the filling of the ventricles.

Henderson,[12] however, in his extensive studies of volume changes of the ventricles of the dog's heart, has reached very different conclusions concerning the function of the auricles. According to him, the auricles, as contractile organs, are of very minor importance; and the auricular systole, when it does force blood into the ventricles, propels a few drops at the most.

Henderson's method for studying volumetric changes of the ventricles briefly stated is as follows: The heart was exposed by opening the thorax and pericardial sac, and was then inserted up to the auriculo-ventricular junction, into a special type of cardiometer. A rubber dam curtain served as an air-tight joint at the auriculo-ventricular junction. The cardiometer was connected by rubber tubing to a Marey tambour which recorded the volume changes; a down stroke indicating a decrease in ventricular volume, and an up stroke an increase in volume.

The volume curve obtained is composed of a sharp descent, representing ventricular systole, an ascent as sharp and abrupt corresponding to the filling of the ventricles or ventricular diastole, and a line parallel to the abscissa representing the period of rest or diastasis. According to Henderson the volume changes of the ventricles occur only during the phases of ventricular systole and ventricular diastole. During the intervening period the ventricular musculature has stopped relaxing. The venous pressure and auricular systole, the forces tending to fill the ventricles, are not sufficient to stretch the ventricular walls, and hence no volume changes occur during ventricular diastasis.

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[8] HÜRTHLE: *Loc. cit.*

[9] FRANÇOIS-FRANCK: Archives de physiologie, 1890, pp. 405-410; Reference, TIGERSTEDT, Physiologie des Kreislaufes, p. 100.

[10] ERLANGER: Journal of experimental medicine, 1905, vii, p. 689.

[11] ERLANGER: This journal, 1906, xvi, p. 163.

[12] HENDERSON: This journal, 1906, xvi, p. 325. A further review of literature on the filling of the ventricles will be found here.

The measurement of volume changes of a rapidly beating ventricle is a very difficult problem, and before we can accept the conclusions of any work based upon plethysmographic records we must be sure of two points:

First, that the apparatus used is delicate enough to register all changes without in any way interfering with the action of the heart.

Second, that the volume changes between the outer walls of the ventricles and the cardiometer furnish a reliable and accurate index of the volume changes going on within the ventricles.

The discussion of the second point will be taken up farther on.

Concerning the delicacy of Henderson's apparatus, Straub[13] points out many vital faults. The mass of the recording tambour is too great to insure delicacy, and the period too slow to record quick changes. Straub also points out that the tension of the rubber is too great and that it varies in different phases of the ventricular cycle.

A recording mechanism, which is to measure the resultant effect of a weak force, such as venous pressure, on the slowly relaxing thick walls of the ventricles should not vary in delicacy during a ventricular cycle, especially not in the wrong direction. With an increase in ventricular volume it would appear that there would be an increase in intra-cardiometer pressure. If after diastole this pressure is just sufficient to counteract venous pressure, then the volume curve should take the form shown in Fig. 9, Henderson.[14] If, on the other hand, this pressure is less than venous pressure, and ventricular relaxation is not yet complete, then ventricular volume should increase at a rate dependent upon venous pressure and muscular relaxation.

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[13] STRAUB: Journal of physiology, 1910, xl, p. 378. (All references to STRAUB refer to this paper.)

[14] HENDERSON: *Loc. cit.*, p. 337.

Some of Henderson's figures, according to our interpretation, show a marked increase of ventricular volume during diastasis. For example, in Fig. 7,[15] the volume increase after diastole mounts to 31.3 per cent of the entire volume change, and in Fig. 10[16] to 32 per cent.

Straub, in repeating Henderson's work, reduced the mass, inertia, and tension of the recording instrument almost to a minimum. He inserted the cat's heart up to the auriculo-ventricular junction into a cardiometer composed of a thistle tube and a thin fenestrated rubber curtain. The cardiometer was connected with funnels of varying sizes depending upon the size of the heart studied. The volume changes were recorded by photographing the fluctuations of a soap film stretched across the mouth of a funnel, and volume curves constructed from the data thus obtained.

Straub found the ventricles to decrease rapidly in volume during ventricular systole, and to increase as rapidly during diastole, so far agreeing with the results of Henderson. But he also found a marked increase of ventricular volume during diastasis due to venous pressure, and a sudden increase synchronous with auricular systole. Straub divides the ventricular cycle into three periods: ventricular systole, elastic diastole, and passive diastole.

The results obtained conform with the general notion concerning the filling of the ventricles, but even with the delicate methods employed by Straub quantitative results cannot be secured. The data obtained do not tell us of the relative importance of venous pressure and auricular systole, for reasons which will now be considered.

The ventricles form a cone-like mass with a large and broad base. The side walls are composed of heavy thick musculature. The base, when the chambers are closed, is composed largely of the thin flaccid auriculo-ventricular valves. When the internal pressure is increased within a water-tight structure, the structure always gives way at its weakest point. There is no question that the auriculo-ventricular valves would be first to give way before an increasing intraventricular pressure. That they do give way during ventricular systole has long been noted. However, pressure, or at least a large pressure, is not absolutely essential to produce bulging of the valves. During relaxation, when blood is flowing into the ventricles the auriculo-ventricular valves float into position. Their final position before auricular systole is dependent upon the rate of venous flow, the length of the chordæ tendinæ, and the relaxation of the papillary muscles. If all of these factors are large, there may be an extreme bulging of the auriculo-ventricular valves; varying any one or all, varies the position of the valves, and since none of these factors can be taken into account in experimental work, the plethysmographic method, as used by Henderson and Straub, can give only approximate results. To mention some cases in point:

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[15] HENDERSON: *Loc. cit.*, p. 331.

[16] HENDERSON: *Loc. cit.*, p. 340.

Straub's records show that during the presphygmic period the volume of the ventricles may apparently increase or decrease.

"The time interval between auricular contraction and the opening of the semilunar valves is occupied by the closure of the auriculo-ventricular valves, and by the 'Anspannungszeit.' To the mechanical disturbances produced by the events is due probably the somewhat irregular part of the curve at this point. The intraventricular pressure may bulge the valves a little toward the auricles and thus cause an apparent diminution in the volume of the ventricle as in Fig. 5 (Straub). The action of the papillary muscle contracting before the fibres of the heart's wall, as we know, may pull the valves toward the ventricles and thus cause an apparent increase in volume, Fig. 4 (Straub). Or both mechanisms may be balanced, and this is perhaps the regular occurrence, as in Fig. 3 (Straub)."

In Fig. 5 (Straub) according to our interpretation the ventricular volume decreased 14 per cent, before the semilunar valves opened. Apparently the auriculo-ventricular valves bulged out of the recording limit of the cardiometer during ventricular systole. But the question arises, when did the valves first bulge? If they bulged first at auricular systole, then the venous flow was recorded, the auricular systole flow only partly recorded. If they bulged during venous flow, then none of the volume changes were correctly recorded.

In Fig. 4 (Straub) the apparent increase in volume during the "Anspannungszeit" was 11 per cent of the entire volume output.

In Fig. 7 (Straub) oscillations were produced by auricular systole amounting to 33 per cent of the total volume changes. Since Straub points out that they are not oscillations of the recording instrument, they can be due only to two things: (1) changes in shape of the base of the ventricles, (2) shifting of position of the cardiometer. Whatever the cause may be, the great danger of using results afforded by the plethysmographic method is clearly indicated.

Other data pointing to this danger were obtained by Henderson.[17] He finds that the coefficient of ventricular discharge varies inversely with the size of the animal to an astonishingly great degree; for a dog weighing 8 kilos the coefficient amounted to 2.6 c.c. per kilo weight, and for a dog weighing 16 kilos it amounted to 1.3 c.c. Henderson also finds that the adaptability of the ventricles accompanying a change of rate from 90 to 180 beats per minute is surprisingly small. No ventricular cycle can be taken as a standard, and if a particular cycle is taken it can lead only to wrong conclusions.

METHOD I.

In passing to the consideration of the method employed in this research it might be well to mention that no attempt is made to determine the quantitative volumetric changes in the ventricular output, but rather to study the end effect (namely, the effect on the arterial blood pressure) of auricular systole, which, after all, is the important factor.

In brief the method employed was to secure independent action of the ventricles and auricles by crushing the auriculo-ventricular bundle, thus rendering the ventricles free of auricular stimuli; then to throw the auricles out of action, for varying periods of time, by fibrillation, while observing the effects on blood pressure.

Dogs the size of fox terriers were used. They were anæsthetized with morphine and ether, tracheotomized and given artificial respiration. Arterial blood pressure was recorded with a mercury manometer. The mammary arteries were tied, the thorax opened, and the heart exposed. The Erlanger heart clamp was then inserted in the usual manner, and the auriculo-ventricular bundle crushed. When it was apparent that complete heart block had been permanently established, the clamp was removed so as to insure free action of the heart valves. After every experiment the heart was opened and carefully examined, and only such experiments were used in this research in which valvular action had not been interfered with.

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[17] HENDERSON: This journal, 1909, xxiii, pp. 359 and 360.

The movements of the auricles were recorded by air transmission, the attachment being made to the appendage of the right auricle by means of an Engelmann's pinzette and tinsel. The auricles were stimulated through the tinsel, by the unipolar method, and thus thrown into fibrillation.

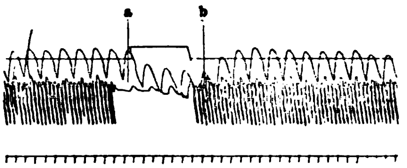


FIGURE 1. — Showing blood pressure changes produced by auricular fibrillation. The upper curve shows arterial pressure and ventricular rate. The line passing through it, the moment of auricular stimulation. The lower curve represents auricular contractions. Auricular rate before and after fibrillation — 156 per minute. Ventricular rate 55 per minute (constant). The blood pressures before, during, and after fibrillation are 50, 40, and 50 mm. Hg respectively. The time of auricular fibrillation is set off on the blood pressure curve by lines *a* and *b*. Time is marked in seconds.

The rate of the ventricles was controlled through stimulation by the unipolar method, using only minimal break shocks furnished by a stimulus selector, the ventricular pulse rate indicating the rate of stimulation. The time was marked in seconds.

The auricles were set into fibrillation for short and long periods of time, with the ventricles beating at various rates. This method of eliminating the pumping action of the auricles was chosen in preference to vagus stimulation, for the reason that it has no effect upon the ventricles. In each case the auricular and ventricular rate and arterial blood pressure were noted before, during, and after fibrillation. If the auricular rate is the same after and before fibrillation, and the ventricular rate is constant, any blood pressure changes must be attributed to the cessation of the pumping action of the auricles.

Fig. 1 is a typical record of blood pressure changes produced by a short period of auricular fibrillation, in a heart with a slow ventricular and a rapid auricular rate. The auricular rate before and after fibrillation was the same, the ventricular rate also remained constant. It will be noted that before auricular fibrillation the blood pressure maintained a level of 50 mm. Hg, but during fibrillation fell suddenly to 40 mm. Hg, a fall of 20 per cent. When the auricles began to beat again, the blood pressure mounted as fast as it fell to its original value.

Fig. 2 shows blood pressure changes produced by long periods

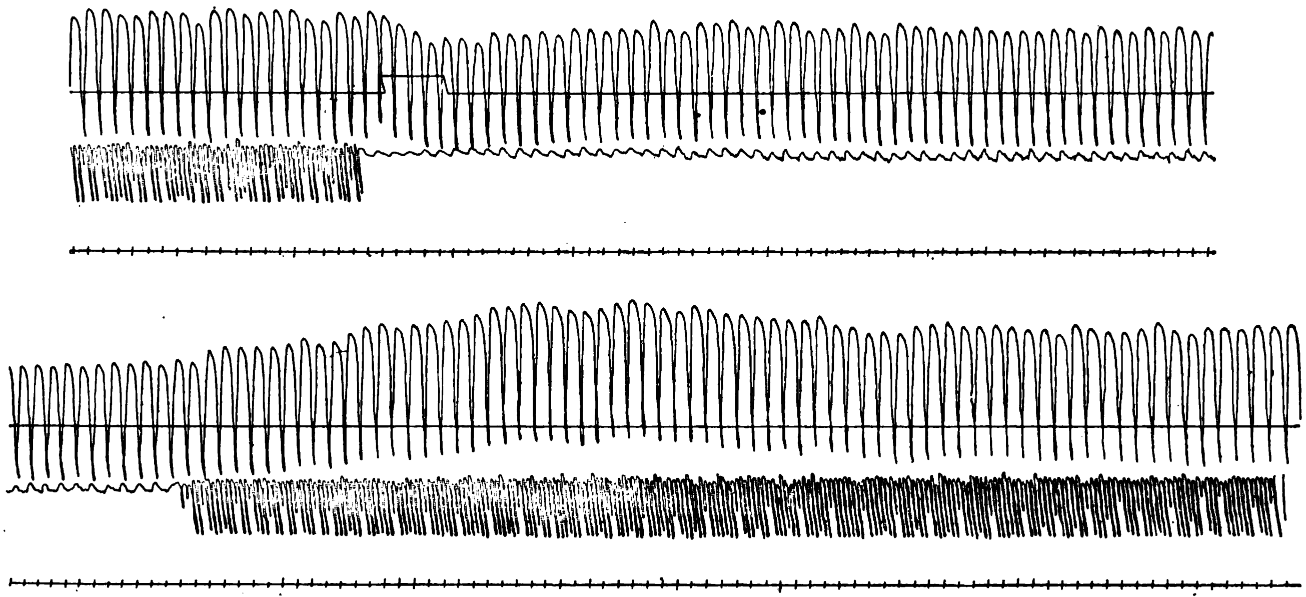


FIGURE 2. — In general the same as Fig. 1. The period of auricular stimulation lasted five seconds, and auricular fibrillation nine minutes and twenty seconds. The auricular rate before and after fibrillation was 180 and 189 respectively. The ventricular rate remained practically constant — 56 per minute. The fall in pressure at the beginning of auricular fibrillation, and the rise at the close of fibrillation amounted respectively to 12 and 24 per cent of their preceding pressures.

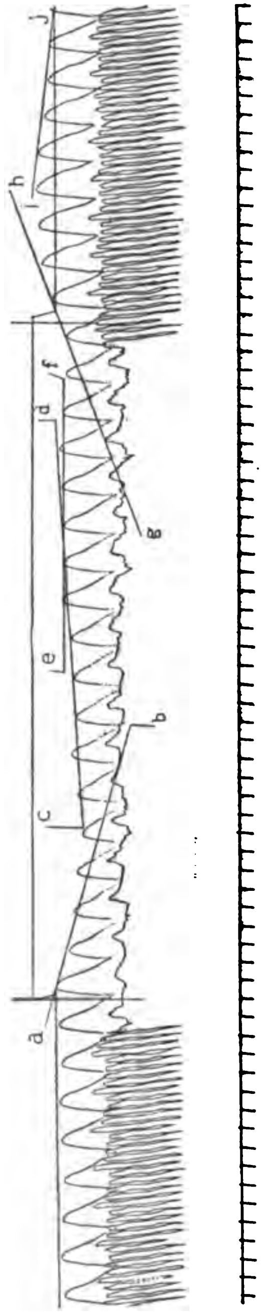


FIGURE 3.

of auricular fibrillation. Before fibrillation the mean arterial pressure maintained a level of 67 mm. Hg. During fibrillation the arterial pressure fell — first abruptly, then more gradually, reaching the lowest level of 59 mm. Hg at the end of the seventh ventricular beat. The pressure then gradually mounted to 63 mm. Hg and remained there for nine minutes. When the auricles beat again, there was a prompt rise in arterial pressure. For twenty-two ventricular contractions the pressure rose to its maximum of 78 mm. Hg. The post-fibrillating increase in pressure amounted to 24 per cent of the preceding pressure, .and the fall amounted to only 12 per cent of the preceding pressure. These large post-fibrillating pressure changes were obtained only when the auricles fibrillated for a long time.

We have referred to the general changes in arterial pressure which prevail when the auricles are thrown into fibrillation. It may be well now to consider these changes more in detail. As mentioned (see Fig. 3) in connection with Fig. 2, so in this case the blood pressure previous to auricular fibrillation remains at a constant level, but the moment the auricles cease to contract, the blood pressure falls rapidly along line *A B*. The fall does not end abruptly, but rounds off gently, reaching the minimal level during beats 5, 6, and 7; and then rises very gradually along line *C D* to a new level, *E F*. This level is maintained as long as the auricles fibrillate. When the auricles beat again, the pressure mounts rapidly along line GH, until presumably the superabundance of blood, which has accumulated during fibrillation, is pumped from the veins. The pressure then rises more gradually and finally sinks along line HJ to the level maintained before auricular fibrillation.

The shapes of the curves representing pressure changes are probably determined as follows: Given a venous pressure, stoppage of auricular contraction produces a definite fall of pressure, indicating the amount of arterial pressure due to the injection of blood into the ventricles by the auricles. As fibrillation continues, the inactivity of the auricles allows blood to accumulate in the veins with the production of a considerable venous pressure, which tends to compensate for auricular failure, increasing in importance with the increase of venous pressure. But a balance is soon reached at which arterial pressure no longer increases. In many cases the increase of venous pressure alone brings the arterial pressure up to the level maintained before auricular fibrillation, but probably only at a very great strain to the venous system. This illustrates a very important function of the auricles, namely, an adequate filling of the ventricles with a comparatively low venous pressure — thus preventing a continued strain upon the venous system.

The pressure changes due to fibrillation of the auricles induced while the ventricles are beating rapidly are the same as those described above (see Figs. 4 and 5; also see Table A, for results with varying relative rates of auricles and ventricles).

METHOD II.

Figs. 4 and 5 show large blood pressure waves (artificially produced by regulating the rate of ventricular contraction). These were first noted when an endeavor was made to produce as normal heart action as was possible under the given conditions, by keeping the ventricles beating by means of electrical stimulation at approximately the same rate as the auricles. When the ventricles in this way were made to closely follow the auricular rate, blood pressure waves similar to those in Figs. 4 and 5 were produced. The explanation of these waves is analogous to that used for beats in the physics of sound, namely, they are the result of the interference of two elements in varying phases of activity. The two physiological elements are the auricles and ventricles. As in sound slightly varying, the difference in rate of vibrations of two tuning forks of approximately the same pitch varies the number of beats per unit of time, so it is with

FIGURE 4

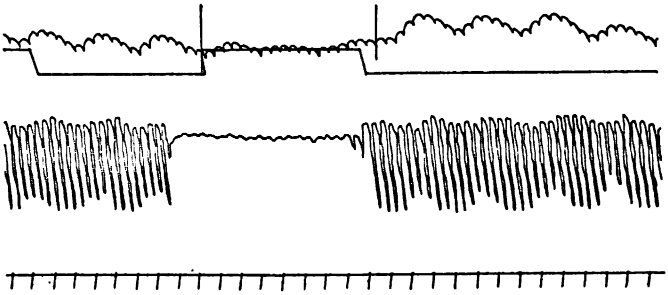
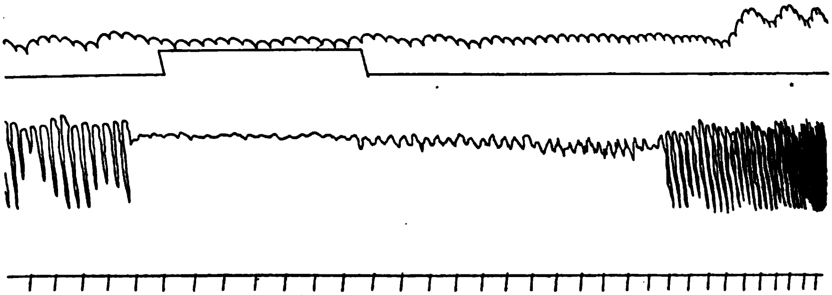


FIGURE 5



FIGURES 4 and 5. — Showing arterial pressure changes produced by auricular fibrillation, with high ventricular rate — auricular rate 162, and ventricular rate 132 per minute. Note the large rise of arterial pressure when auricles resume their function.

the heart under the given conditions. When the difference between auricular and ventricular rate was increased, the interference waves shortened, and the number of systoles per wave diminished. On the other hand, decreasing the difference increased the length of the interference waves and the number of systoles per wave. In every interference wave the number of auricular and ventricular beats differed by one. Therefore it is evident that the faster cardiac element falls back one complete cycle of the slower element. The rate at which the faster element shifts its position depends entirely upon the length of interference wave produced. It became apparent that

TABLE A.

Number of experiment.	Auricular rate per minute.	Ventricular rate per minute.	Mean arterial pressure in mm. Hg.	Pulse pressure in mm. Hg.
1 *b*[1]	210	57	66	48
d	F	58	63	42
a	198	57	69	50
2 *b*	198	56	67	47
d	F	57	59	42
a	192	56	78	52
3 *b*	192	51	68	49
d	F	51	63	42
a	188	51	72	52
4 *b*	156	35	50	18
d	F	35	40	17
a	156	35	50	19
5 *b*	188	78	66	. .
d	F	78	59	. .
a	180	78	65	. .
6 *b*	162	138	62	. .
d	F	138	56	. .
a	162	138	66	. .
7 *b*	174	150	60	. .
d	F	144	60	. .
a	168	144	65	. .
8 *b*	162	132	62	. .
d	F	132	59	. .
a	162	132	70	. .
9 *b*	162	132	65	. .
d	F	132	59	. .
a	162	132	70	. .
10 *b*	162	132	62	. .
d	F	132	60	. .
a	162	132	68	. .

[1] *b*, *d*, and *a* refer to periods immediately before, during, and immediately after auricular fibrillation.

the production of interference waves offered a very good opportunity of studying, not only the importance of auricular systole, but also its efficiency in various parts of ventricular cycle.

Figs. 6, 7, and 8 show interference waves of varying lengths. Note that waves of the same contour are produced whether the auricles or ventricles have the quicker pace. (For further description of similar curves see Table B.)

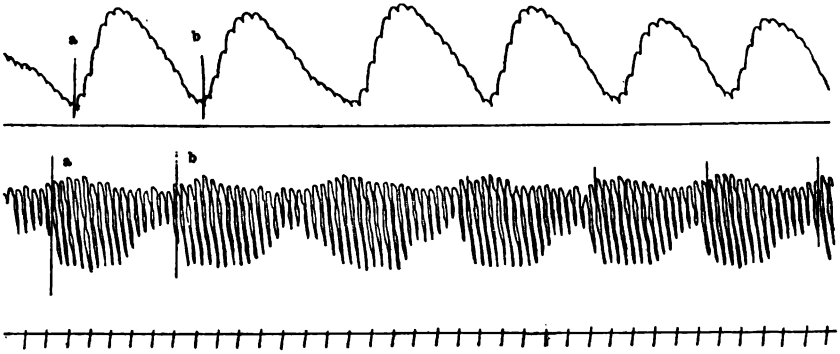


FIGURE 6. — Showing interference waves, each with 16 ventricular and 17 auricular cycles. The arterial pressure rises for 6 ventricular systoles.

a. The method of study now turned upon the production of interference waves of varying lengths to determine the efficiency of auricular systole at different moments in the ventricular cycle. The Hürthle spring manometer was substituted for the mercury manometer in the hope that the auricular systoles might be recorded in the blood pressure curves, and that the tracings might bring out some points hidden by the inertia of the mercury manometer. The striking feature of the Hürthle tracing is the great changes of pulse pressures during different phases of interference. During the stoppered action of the auricular systoles the pulse pres-

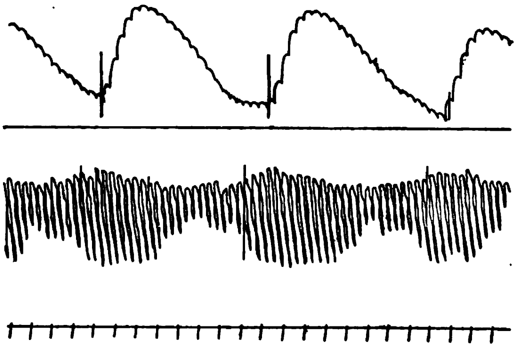


FIGURE 7. — Showing interference waves, each with 23 ventricular and 22 auricular cycles. The pressure rises for 6 ventricular systoles.

TABLE B.

Auricle and ventricle.	Interference wave number.	Auricular rate per minute.	Auricular and ventricular beats per wave.	Arterial pressure in mm. Hg.	No. of ventricular beats during rise of arterial pressure.	No. of ventricular beats during fall of arterial pressure.
a	1	174	22	54	6	17
v	..	...	23	84		
a	2	174	17	54	6	10
v	..	...	16	82		
a	3	174	14	60	5	8
v	..	...	13	80		
a	4	174	8	64	3	4
v	..	...	7	70		
a	5	174	5	54	3	3
v	..	...	6	58		
a	6	174	4	60	2	3
v	..	...	5	63		
a	7	168	9	74	3	5
v	..	...	8	82		
a	8	168	9	74	3	5
v	..	...	8	82		
a	9	168	23	62	7	15
v	..	...	22	76		
a	10	168	13	68	5	9
v	..	...	14	80		
a	11	168	16	74	8	9
v	..	...	17	84		
a	12	168	15	80	7	9
v	..	...	16	120		

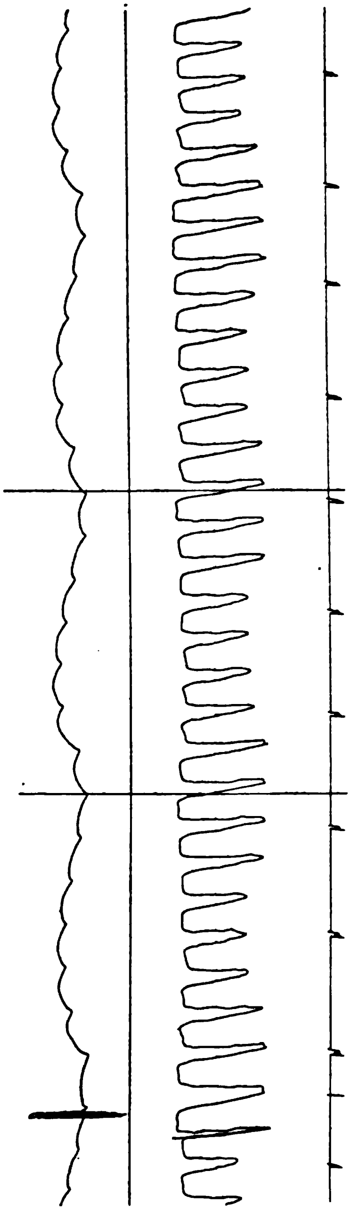


FIGURE 8. — Interference waves, each with 7 ventricular and 8 auricular cycles.

sure amounted to hardly 40 per cent of the pulse pressure when the auricles and ventricles were beating in proper sequence (Fig. 9). If pulse pressure is an index to volume output,[18] we have here very good evidence of the relative amount of blood forced into the ventricles by a properly placed auricular systole.

b. On failure to secure any definite information as to time relations between auricular systole and ventricular systole with the Hürthle manometer, the effort was made to secure a result through recording intraventricular pressure. A trochar was inserted through the walls of the right ventricle in order not to interfere with valvular action. The pressure changes were recorded with a delicate sphygmoscope. The arterial pressure was again recorded with the mercury manometer. It was hoped that every auricular impulse would show distinctly upon the intraventricular pressure curve, and in that way give proper time relations of auricular and ventricular systoles, as well as tension changes within the ventricles during ventricular diastole. The recording mechanism, however, was not of sufficient delicacy; therefore in many places (see Fig. 10) the auricular systole failed to leave an impress on the curve, and in other places the auricular rise ran imperceptibly into the rise due to ventricular systole. The usual form of intraventricular pressure curve obtained shows a sharp systolic rise due to ventricular systole, followed by a long curve of relaxation, which in turn is followed by a rather sharp shoulder (*a*).

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[18] DAWSON and GORHAN: Journal of experimental medicine, 1908, p. 484.

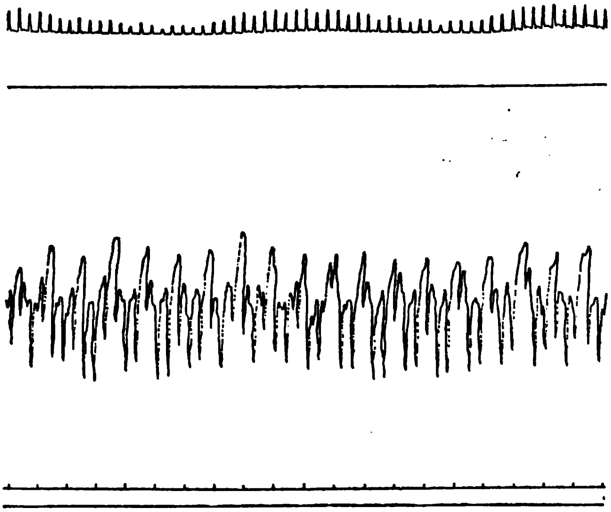

FIGURE 9. — Interference waves recorded with Hürthle Manometer showing large changes in pulse pressure (ventricular output).

This shoulder is probably produced by venous flow after ventricular diastole. The effect of auricular systole is seen mainly upon the shoulder mentioned, and upon the amplitude of the systolic rise. If auricular systole occurs at the proper time, the rise of the shoulder is continued as a straight line, into the ventricular systolic rise, which in this case is very high. A high systolic rise, it seems, would indicate a greater ventricular filling, with a greater ventricular output, forced from the ventricle in approximately the same time required for smaller outputs. A group of three intraventricular pressure curves above the bracket shows very beautifully the importance of the proper placing of auricular systole in the ventricular cycle. With the advancing of the auricular systole to a more favorable moment of contraction the intraventricular pressure is increased. In the first ventricular contraction the preceding auricular systole occurred early in diastole and natur-

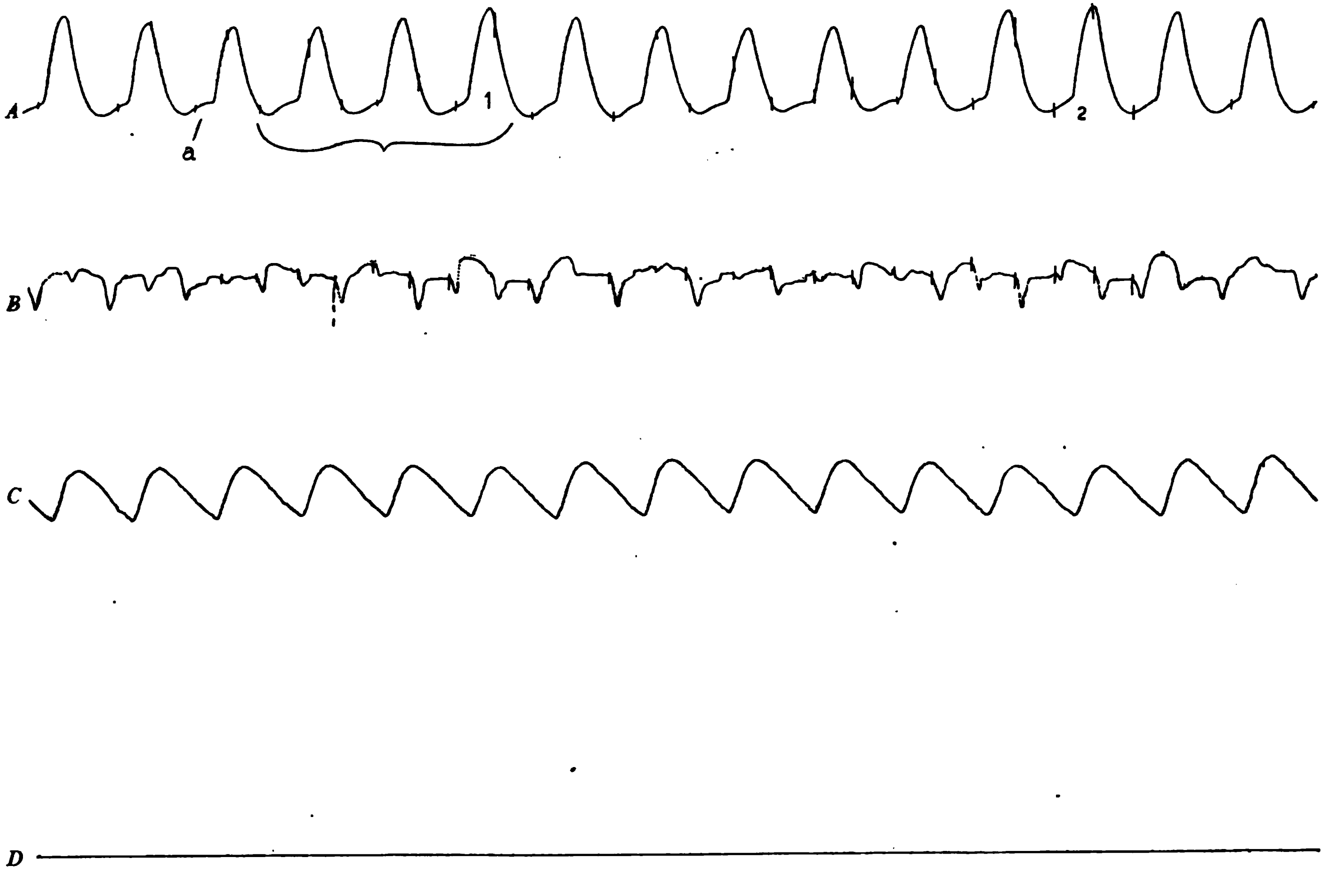

FIGURE 10. — Showing effects of differently placed auricular systoles on intraventricular pressure curves. *A*, Intraventricular pressure; *B*, Auricles; *C*, Arterial pressure: *D*, Base line.

ally was not very effective. In the second contraction auricular systole advanced to a more favorable position; occurring immediately before ventricular systole, but a trifle too soon to allow complete auricular emptying. The auricles were stoppered by ventricular systole. For the third ventricular contraction the auricular systole occurred at the optimum moment; the auricles were completely emptied at the beginning of ventricular systole. In this case the maximum intraventricular pressure was reached. During the three ventricular systoles described the venous pressure could hardly have changed. The period of ventricular relaxation remained constant. The only factor that varied was the placing of auricular systole in the ventricular cycle.

c. To obtain accurate relation between auricular and ventricular systoles during various phases of interference waves the movements of the ventricles were recorded in exactly the same way as those of the auricles. The Engelmann's Herzpinzette was attached to the bulging part of the right ventricle. (The time relation of the auricles and ventricles can be measured to any degree of fineness, depending upon the rate of the moving surface.) At the same time venous pressure was recorded by means of a water manometer, the side pressure of the innominate vein was measured by inserting an ordinary washout cannula into a large innominate branch, just at the point of origin. Only relative venous pressures were measured.

For general results obtained with the above method see Fig. 11, which is a record of a short interference wave on a relatively slow drum. It will be noticed that all the tracings show a wavelike rhythm. The amplitudes of the auricular systoles vary in a perfectly regular manner. The significance of this is very important. It will be noticed that the short beats occur wholly or partly during ventricular systole, at a time when the auriculo-ventricular valves are closed and block the blood impelled against them by auricular contraction. On the other hand, the auricular systoles of high amplitude fall into the ventricular cycle during some phase of ventricular relaxation. The auricles are then not opposed by the ventricles and are free to empty themselves into them. It is evident, then, that the amplitude of auricular systole furnishes for our purposes a valuable index of the amount of blood forced into the ventricles with each auricular systole. The auricular systoles of increasing amplitude mark a shifting of auricular

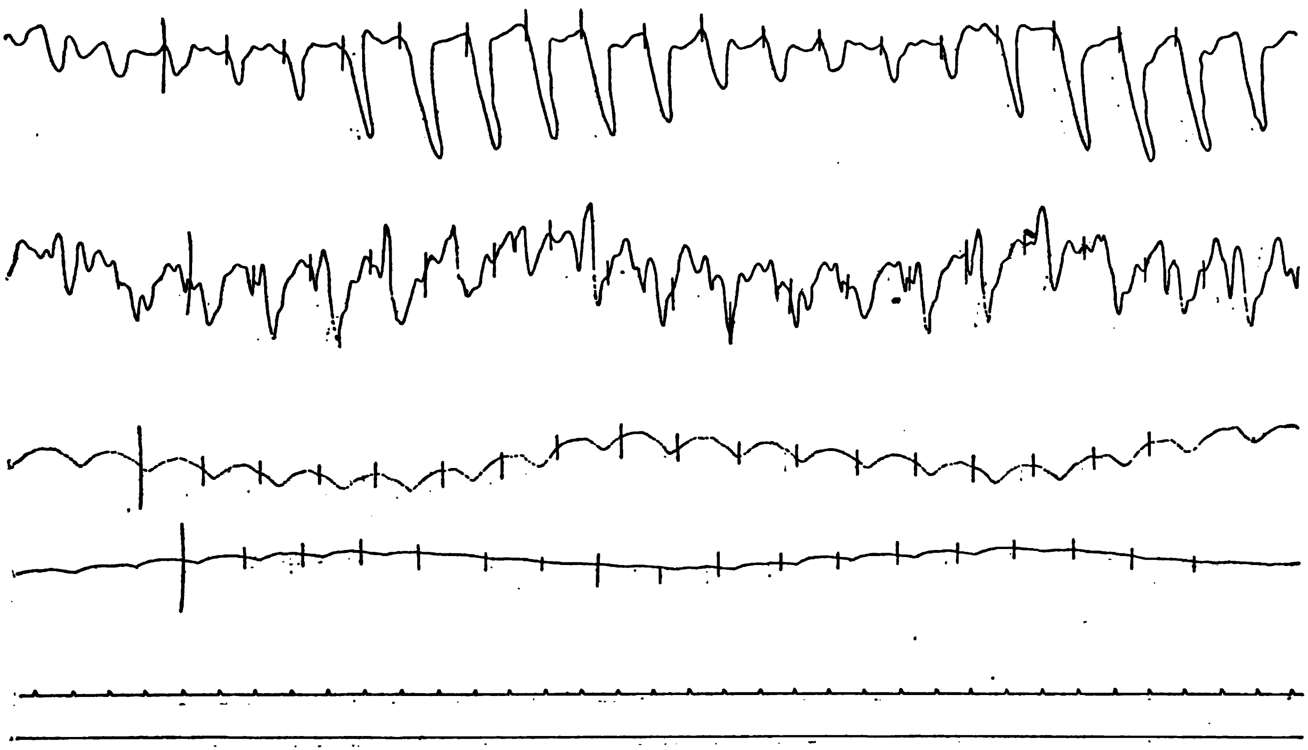

FIGURE 11. — Interference waves showing roughly the time relation of auricular and ventricular systole, and also venous pressure changes. The curves from above down represent auricular and ventricular contractions, arterial pressure and venous pressure. Time is marked in fifths of seconds.

systole favorable for auricular support, and those of decreasing amplitude an unfavorable shifting to a moment when no blood is forced into the ventricles. One important point must be borne in mind, namely, that the maximum amplitude does not necessarily indicate the auricular systole most efficient in producing the largest end result, ventricular output. For it is apparent that the auricles can empty themselves just as well or better when the auricular systole occurs during the latter part of ventricular diastole, or the first part of a diastasis before the blood has accumulated in the ventricles through venous flow, than later in diastasis. That an auricular systole placed in ventricular diastole, or early in diastasis, is comparatively inefficient will be shown further on.

Venous pressure changes naturally are produced by interference with auricular output. In the period in which the auricles are stoppered the venous pressure rises, and in distinct waves, indicating clearly that the auricles are meeting with resistance. As soon as the auricles are free to force onward the blood, which has accumulated in the large veins, the venous pressure falls, the waves now being less distinct, indicating that the auricles are meeting with less resistance.

During the rise of arterial pressure the auricular systole is gradually shifting to the most favorable moment of contraction eventually to pass that point to a less favorable moment. As will be pointed out, the moment of maximum arterial pressure does not mark the optimum relation of auricular systole to ventricular systole. The optimum $A^s V^s$ interval occurs at some point on the arterial rise.[19]

For purposes of studying more accurately the auriculo-ventricular relation, long interference waves were produced and recorded on a rapidly moving surface. Long waves were chosen so that a more minute analysis would be possible.

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[19] WENCKEBACH: Deutsches Archiv für klinische Medicine, 101, iii–iv, p. 411. WENCKEBACH noticed a condition similar to that described here in a case of paroxysmal tachycardia in man. The arterial pressure was very low, the venous pressure high, with a marked venous pulse. The heart rate was 240 per minute. There were all the signs of a vigorously beating heart, yet the arterial pressure was low. The symptoms only accompanied a high heart rate, and could be made to disappear by compression of the vagus. WENCKEBACH explains the condition as a "Pfroffung" of the auricles caused by synchronous systoles of auricles and ventricles. The blood cannot pass into the ventricles as fast as it accumulates in the veins, producing a venous stasis.

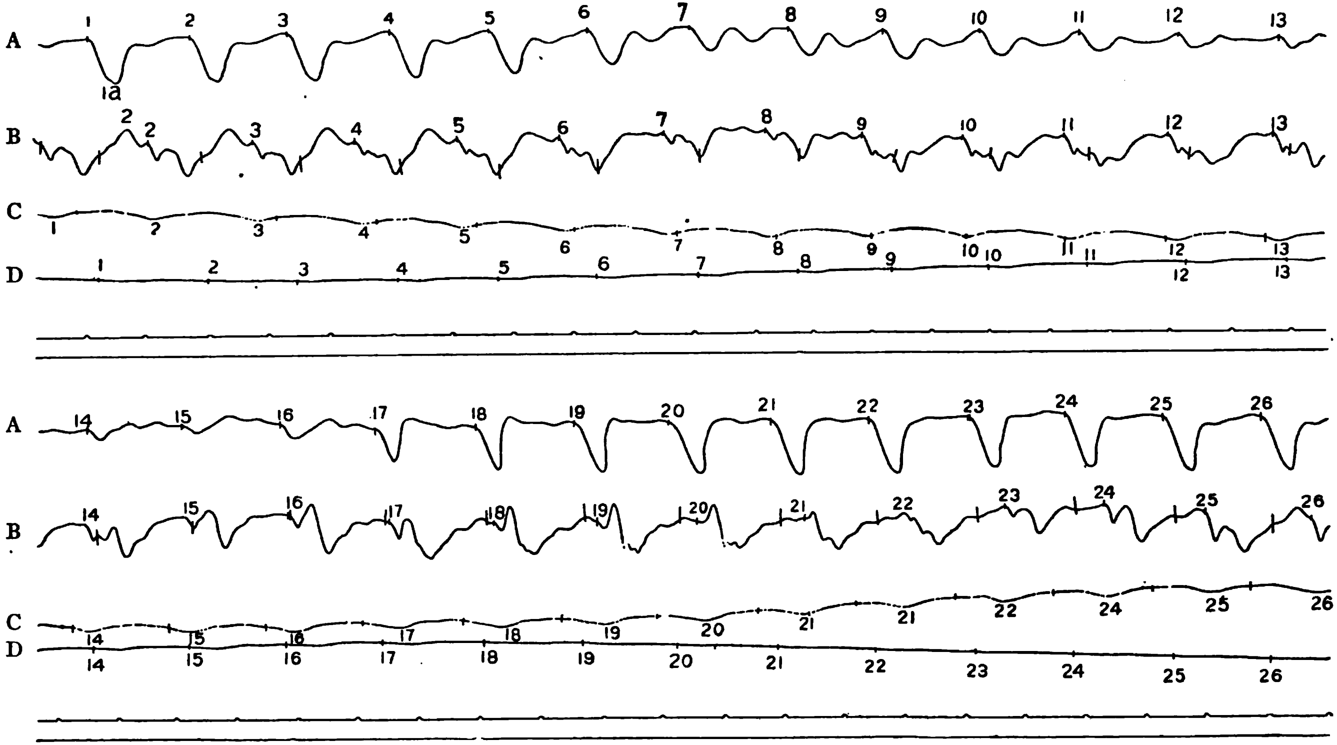

FIGURE 12. — Same as Fig. 11. Recorded on rapidly moving surface. The auricular systoles are numbered at the beginning of contractions, which point is set off on the ventricular curves. The ventricular systoles are also numbered at the beginning of contraction. The curves from above down represent auricular and ventricular contractions, arterial pressure and venous pressure. Time is marked in fifths of seconds. (For further description see Table C.) *A*, Auricles; *B*, Ventricles; *C*, Arterial pressure; *D*, Venous pressure.

Fig. 12 shows such a wave, recorded on a drum revolving at a speed of 8.3 cm. per second. There are 25 auricular systoles and 24 ventricular systoles per wave. Each auricular systole shifts back on the ventricular cycle 1/24 of that cycle. It will be noticed, in the table accompanying Fig. 12, that the duration of the ventricular cycle is 0.379 second. The shifting per auricular beat amounts therefore to 0.015 of a second. Time is marked in fifths of seconds. The space between time marks allows of the determination of time relations in seconds to the third decimal place.

To understand Fig. 12 refer to Table C, page 54. Auricular systole 1 occurs at the moment of highest arterial pressure and forces blood into the ventricles preparatory for ventricular systole 2. Judging from the amplitude of the auricular contraction, a large amount of blood was forced into the ventricles. Six preceding auricular contractions show as large an amplitude. In spite of the fact that auricular systolic output is almost maximal, auricular systole 1 marks the turning point for arterial pressure. From this point on arterial pressure falls steadily. This fall can hardly be attributed only to the amount of blood (amplitude of auricular systole) forced into the ventricles by auricular systole 1. It seems probable that the amount of auricular contraction also plays a part. Auricular systole 1 occurred 0.011 second after the abrupt ventricular relaxation, bringing it early in ventricular diastasis. As will be shown later, this is an unfavorable moment to produce a large ventricular output. The A^sV^s interval is too great.

As is shown by notch *a* at the bottom of the down stroke, auricular systole 1 is the first contraction of the auricle to meet with any marked resistance. The ventricles have relaxed sufficiently to make room for only part of the auricular discharge. The auricles, therefore, meet with resistance only at the end of the auricular systole, when the ventricles need stretching to allow the entrance of more blood. Auricular systole 2 shifts further back in ventricular cycle 3 and therefore meets with resistance earlier, as is shown by the backward movement of notch *a*, and the shortening of auricular amplitude. With every subsequent auricular systole the moment of contraction becomes more and more unfavorable, the auricles meet with increasing ventricular opposition, and therefore inject less and less blood into the ventricles. The ventricular output gradually diminishes with an accompanying fall of arterial pressure.

TABLE C.

Auricular systole. Number.	Ventricular systole. Number.	Arterial pressure in mm. Hg corresponding to ventricular systoles 1–26.	Change in arterial pressure due to auricular systole in same line.	Difference in venous pressure in mm. H_2O.	Venous pressure.	Phase of venous pressure.	Phase of arterial pressure.	Remarks.
1	2	84.0	2.5	1.2	46.0	↓	↓	Ventricular rate, 180 per min.
2	3	81.5	1.9	0.5	44.8	│	│	Beats per wave, in 8 sec. Auricular 25
3	4	79.6	2.4	0.0	44.3	↓	│	Ventricular . . . 24
4	5	77.2	2.0	0.0	44.3	↑	│	
5	6	75.2	1.4	0.7	45.0	│	│	Time of Cycles: Aur'lar Systole 0.0631
6	7	73.8	3.2	1.4	46.4	│	│	Aur'lar Diastole 0.0737 } 0.326 sec.
7	8	70.6	1.6	1.6	48.0	│	│	Aur'lar Diastasis 0.190
8	9	69.0	0.0	1.0	49.0	│	│	
9	10	69.0	0.4	1.6	50.6	│	│	
10	11	68.6	1.6	1.4	52.0	│	│	

11	12	67.0	1.4	1.0	53.0			. . . . .	
12	13	65.6	0.6	1.0	54.0			. . . . .	
13	14	65.0	0.2	1.0	55.0			. . . . .	
14	15	64.8	0.0	0.6	56.6			. . . . .	
15	16	64.8	0.0	1.4	57.0			. . . . .	
16	..	64.8	0.0	0.0	57.0	↑	↓	Vent'lar Systole 0.116	
17	17	65.6	0.8	0.0	57.0	↓	↑	Vent'lar Diastole 0.063	0.379 sec.
18	18	67.0	1.4	0.0	57.0			Vent'lar Diastasis 0.200	
19	19	68.0	1.0	1.2	55.8			. . . . .	
20	20	69.8	1.8	1.6	54.2			. . . . .	
21	21	73.0	4.8	2.2	52.0			. . . . .	
22	22	77.0	4.0	1.6	50.0			. . . . .	
23	23	80.6	3.6	1.4	49.0			. . . . .	
24	24	83.4	2.8	1.5	47.5			. . . . .	
25	25	85.0	2.6	2.0	45.5			. . . . .	
26	26	85.0	0.0	...	...	↓	↑	. . . . .	

Auricular systole 6 is of considerable interest. Referring to Table C it will be noticed that auricular systole and ventricular diastole are of the same duration, 0.063 second. According to Henderson the blood flow into the ventricles is no faster than ventricular relaxation will allow, the diastole is the only period of volume increase of the ventricles, and the auricles are of practically no importance in the filling of the ventricles. But the auricular impulse is of sufficient strength to stretch the relaxing ventricular walls, for if it were not, the auricular amplitude should fall off abruptly, and also the arterial pressure, as in fibrillation. This, however, is not the case. As the part of auricular systole occurring during ventricular diastole shortens, the arterial pressure falls off gradually, and only when the auricular systoles occur *entirely* within ventricular systole does the pressure remain constant.

That the ventricles can be stretched during diastole and filled to a greater extent by auricular systole is shown in Fig. 13, by the interference waves produced in a rapid heart of 600 ventricular cycles per minute. There is no period of ventricular diastasis. The auricles are completely stoppered during ventricular systole, and therefore must be effective in filling the ventricles even in ventricular diastole. To come back to the analysis of Fig. 12.

The group of auriculo-ventricular cycles from auricular systole 6 to 14 represent according to Henderson's theory a condition very similar to the normal filling of the dog's ventricles. The auricular systole being of no significance, the ventricle fills during diastole, leaving the entire period of diastasis uninterrupted. The arterial pressure falls continually from 6 to 14, despite the fact that venous pressure is mounting rapidly, almost reaching its maximum at auricular systole 14.

Ventricular systole is of longer duration than auricular systole, and consequently there occurs a group of auricular systoles (14, 15, and 16), all of which occur entirely within ventricular systole. This period marks a time in which there are no variable factors at work, for the venous pressure has already mounted to a point at which venous pressure and ventricular output are balanced (previously referred to). It will be noticed that at this period the arterial pressure is at its minimum and remains absolutely constant.

Auricular systole 17 is the first systole after complete interference to force any blood into the ventricles. It marks the turning points for both arterial and venous pressures. Auricular systole 17 begins 0.01 of a second before ventricular systole and therefore only one sixth of it is effective in injecting blood into the ventricles, five sixths of it being stoppered by ventricular systole. From auricular systole 17 to 20 inclusive the amplitude of the auricular contractions increases, as a greater and greater part of them becomes effective, and a smaller

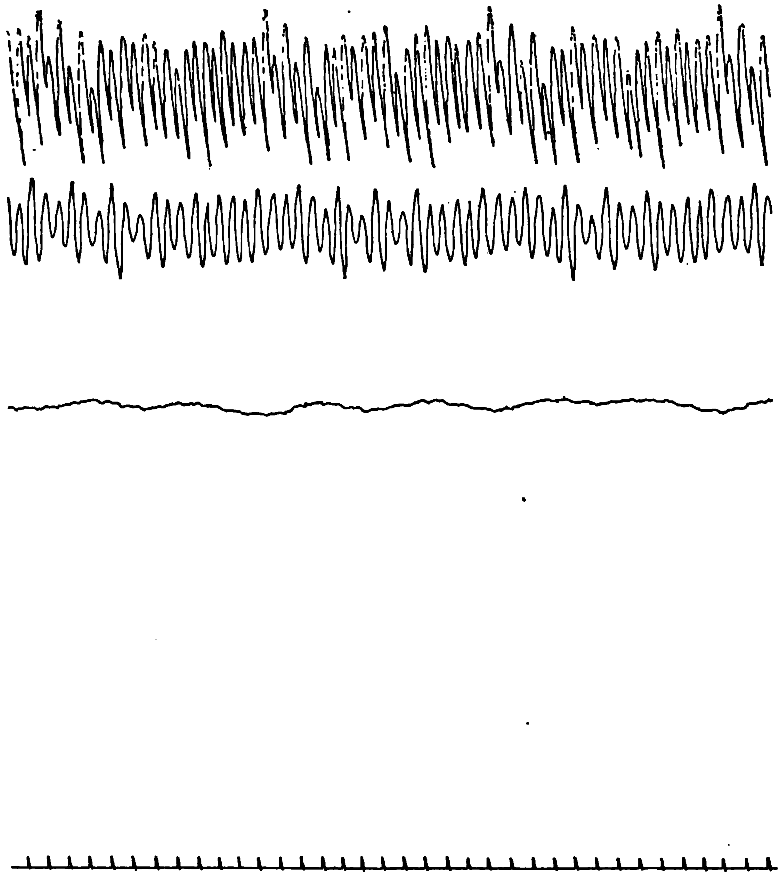

Figure 13. — Interference waves produced by very rapid stimulation of ventricles (600 per minute). Arterial pressure, 120 mm. Hg. Note there is no period of diastasis. The curves from above down represent ventricular and auricular contraction, and arterial pressure. Time is marked in fifths of seconds.

and smaller part becomes stoppered by the ventricles. Note the gradual and steady rise of arterial pressure, which indicates a gradually increasing efficiency of the auricular systoles mentioned. It is of importance to remember that all of this group of auricular systoles are to some extent interfered with.

Auricular systole 21 is the first systole to shift back entirely into ventricular diastasis. It finishes 0.008 of a second before ventricular systole begins, and effects a rise of arterial pressure amounting to an unusual amount of 4.8 mm. of Hg. The following table D gives accurately the auriculo-ventricular time relation and the changes in arterial pressure following each ventricular systole. Fortunately, for some obscure reason the auricular systolic shifting just at this critical moment was very slow, so that the record offers an exceptionally favorable opportunity for determining, with a high degree of refinement, the optimum moment of auricular contraction.

The question now arises how many factors are there at work in the interval of auricular systole under consideration in Table C, causing these large blood pressure changes. Whether the relative amounts of blood forced into the ventricles, and the varied perfection of valvular action alone can account for the changes, is very difficult to say.

The physiological condition of the ventricular muscle at the moment of ventricular stimulation may be of considerable importance. It is a well-known fact that a properly stretched muscle gives a far more efficient contraction to a given stimulus than a relaxed muscle. The same probably holds true with ventricular muscle, without in any way being at variance with the "all or none law." Straub's records clearly show that the tension of the ventricles is markedly increased by auricular systole, and lasts a short but appreciable time. This period of tension may be of considerable importance. Probably that auricular systole producing it just at the moment the ventricular impulse reaches the ventricles is the most efficient systole in producing the largest end result, namely, ventricular output.

After auricular systole 21 the pressure continues to rise. This does not mean that the auricles are increasing in efficiency. The maximum efficiency of an auricular contraction can be measured by its individual effect, which it has been seen is greatest with auricular systole 21. A number of auricular systoles, following 21, although gradually shifting to a more and more unfavorable moment of contraction are still efficient in maintaining a comparatively high ventricular output; and so long as the ventricular output, whether it be increasing or diminishing, remains above capillary flow, the blood pressure in the arteries will rise.

We have analyzed one complete interference wave, and we may now review the effects of auricular systole in different parts of the ventricular cycle with the help of Fig. 14, in which the auricular systoles are divided into groups according to their efficiency, and placed in the ventricular cycle as they occurred in Fig. 12. The efficiency of auricular systole is designated by the lines above the numbered groups.

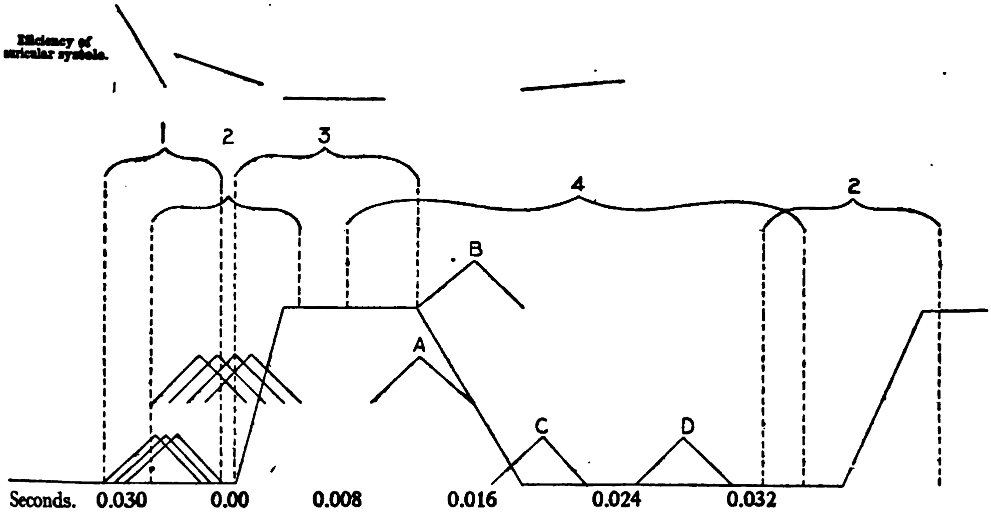

FIGURE 14.

Group 1. Auricular systoles completed very shortly before ventricles begin to contract, have the most marked effect.

Group 2. Auricular systoles occurring partly during ventricular diastasis and ventricular systole have a marked effect.

Group 3. Auricular systoles occurring completely within ventricular systole have no effect.

Group 4. Auricular systoles: *a*, occurring partly during ventricular systole and diastole; *b*, occurring wholly within ventricular diastole; *c*, occurring partly within ventricular diastole and diastasis; *d*, occurring wholly during ventricular diastasis, all have an effect — increasing from left to right — from minimum to maximum.

Concerning the optimum period of auricular systole we are not prepared to give final figures within thousandths of a second. All that can be said is that the best records obtained show that a slight shifting of a few thousandths of a second alters the effectiveness of auricular systole, and that the most effective auricular systole occurred 0.008 of a second before ventricular systole. Unfortunately these best records show time relations of the right heart, while the blood pressure which was used as an index of auricular efficiency is an index to the efficiency of the left auricular systole. Making correction reference for the passage of the impulse from the right to the left auricle, the most effective left auricular systole in Fig. 12 finished just as the ventricular systole began. Numerous records show that the most effective interval mentioned between ventricular systole and completion of auricular systole may be as long as 0.02 of a second. Some records taken on the left side show this interval to be about .01 of a second.

The results are of especial interest in that they show that the time relations between auricular systole and ventricular systole in normal dogs is approximately the optimum. Judging from the records of Stassen, Cushing and Grosh, and Erlanger, in the cardiac cycle of the dog's heart, the auricular systole is completed immediately, or as long as .02 of a second before ventricular systole. It would be of even greater interest to know if the same interval is optimum for hearts of other animals. In man we find considerable difference in the time relations in the cardiac cycle. The auricular systole often is completed approximately 0.08 of a second before ventricular systole.[20] The same relation is shown for the horse in the curves of Chauveau and Marey.

METHOD III.

In this series of experiments the auricles were set into fibrillation in different phases of waves due to interference. When they were thrown out of function at the crest of a wave, or on the rising limb near the crest, the fall in pressure was rapid. When, on the other hand, they were made to fibrillate at the trough of the wave, the pressure was practically unaltered. At or near the crest the auricles are beating with telling effect. The conditions, therefore, are very favorable for a rapid fall of arterial pressure with a failure of auricular activity. The conditions in the trough of the interference wave are entirely different. Here the auricular systoles are of no aid in forcing blood into the ventricles. They are stoppered by the ventricles.

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[20] ERLANGER: Johns Hopkins Hospital bulletin, 1905, xvi.

TABLE D.

Auricular systole. Number.	Completed 0. . . sec.	Before ventricular systole. Number.	Arterial pressure changes in mm. Hg.
21	0.008	21	4.8
22	0.012	22	4.0
23	0.012	23	3.6
24	0.02	24	2.8
25	0.03	25	2.6

Therefore, when the auricles are thrown into fibrillation, conditions effecting ventricular output are not altered. The venous pressure during the descent of the pressure limb is gradually increasing as the auricular systole shifts into a more and more unfavorable moment of contraction, and when the trough is reached venous pressure has already reached the point of balance (line *E F*, Fig. 3).

For the same reason it may be added, the minimum arterial pressure produced by interference of the auricles and ventricles is not as low as that produced by fibrillation of the auricles as in Method I. The interference waves, therefore, do not indicate the full effect of auricular systole upon blood pressure, since the nullification of auricular action is compensated by the coincident rise of venous pressure.

SUMMARY AND CONCLUSIONS.

The methods used by Henderson for studying the volume changes of the ventricles of the dog's heart appear to be inaccurate, and consequently some of the conclusions drawn by Henderson would seem to require reinvestigation.

The plethysmographic method as employed by Henderson and Straub is not suitable for the accurate study of volume changes of the ventricles for the reason that the movements of the base of the heart, its most capacious part, are not taken into account. Plethysmographic records cannot be even relatively correct, because it is impossible to know at any given time the position occupied by the auriculo-ventricular diaphragm.

In order to determine the efficiency of the auricles in the propulsion of blood into the ventricles, the end results, namely, the effect upon arterial pressure, of varying auricular action must be determined.

The methods used to study these end results were to produce independent action of the auricles and ventricles.

Then: *a*. The auricles were made to fibrillate; *b*. The auricular systole was so placed in different parts of the ventricular cycle that interference waves were produced; *c*. The auricles were set into fibrillation in varying phases of interference waves — and in all cases the effect on arterial pressure observed.

Auricular contractions play an important rôle in determining ventricular output.

1. When the ventricles are beating independently of the auricles, nullification of auricular activity resulting from fibrillation causes a fall in arterial pressure amounting to 10 to 15 per cent.

2. An increased venous pressure results from auricular fibrillation, and compensates to a considerable extent for auricular failure.

3. The same high venous pressure will, when the auricles resume their function, produce relatively large ventricular outputs. The auricles then to be most effective should have a large amount of blood at their command.

4. By gradually shifting the time relations of auricular systole with respect to ventricular contractions, arterial blood pressure changes are obtained amounting to 55 per cent of the pressure that can be maintained by the filling action of venous pressure alone.

5. The auricular systole varies in effectiveness in producing ventricular output, depending upon the time it occurs in ventricular cycle (see Fig. 14).

a. Auricular systoles (Group 1) completed approximately 0.008–0.02 of a second before ventricular systole is begun, seem to have the greatest effect.

b. Auricular systoles (Group 2 and 4*a*), partly stoppered by ventricular systole, have a positive effect upon the filling of the ventricles.

c. Auricular systoles (Group 3) occurring entirely within ventricular systole seem to have no filling effect whatever.

d. Auricular systoles (Group 4) occurring partly within ventricular systole, and in ventricular diastole and diastasis, increase in effectiveness from nil to maximum as they approach the beginning of ventricular systole.

This problem was taken up at the suggestion of Dr. Erlanger, to whom I wish to extend my hearty thanks for his kind help and interest throughout this research.

CONTRIBUTION TO THE PHYSIOLOGY OF LYMPH. — XVII. THE SUPPOSED PRESENCE OF THE SECRETION OF THE HYPOPHYSIS IN THE CEREBRO-SPINAL FLUID.

By A. J. CARLSON and L. M. MARTIN.

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IN a series of investigations in this laboratory on the physiology of the lymph with special reference to the internal secretions, immune bodies, and enzymes present in the body fluids, the fact has been brought out that all of these bodies so far studied reach their highest concentration in the serum, or at least a concentration as high as that in any of the other body fluids. The mechanisms by means of which this higher concentration in the serum is effected are not known. These bodies may pass primarily into the lymph in the organs producing them, and the final concentration in the serum may thus be a balance between the rate of production and the rate of destruction; or they may pass directly into the blood, through the capillaries, and then be distributed to the other body fluids in accordance with the law which governs the equilibrium between the serum and the lymph. There is no direct evidence in favor of the first hypothesis. The second hypothesis seems to have been proven for the adrenals by Dreyer's well-known experiment,[1] and for immune bodies in passive immunity by Hektoen and Carlson,[2] and Greer and Becht.[3] Because of these facts we became greatly interested in the recent experiments of Cushing and Goetch,[4] by which they consider that they have substantiated Herring's[5] hypothesis that the secretion of the pituitary body passes directly into the cerebro-spinal fluid, and is thence distributed to the other body fluids. Herring based his hypothesis upon histological studies, the hyaline bodies of the pars intermedia and the pars nervosa seemingly passing straight through the tissues of the stalk and being forced into the infundibular recess. The hyaline bodies appear to be absent in the hypophysis of the elasmobranches.[6] Cushing and Goetch injected concentrated human cerebro-spinal fluid into rabbits and obtained a slight pressure action similar to that obtained from various preparations of the hypophysis. In experiments not yet reported in full, the same authors found that injection of cerebro-spinal fluid diminishes the sugar tolerance.

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[1] Dreyer: This journal, 1899, ii, p. 203.

[2] Hektoen and Carlson: Journal of infectious diseases, 1910, vii, p. 19.

[3] Greer and Becht: This journal, 1910, xxv, p. 292.

[4] Cushing and Goetch: This journal, 1910, xxvii, p. 60.

[5] Herring: Quarterly journal of physiology, 1908, i, p. 281.

It is obvious that the conclusions drawn by Cushing and Goetch from their experiments are open to a number of grave objections. The cerebro-spinal fluid used in most of the experiments was pathological, and was concentrated twenty to twenty-five times, and the injections intravenously were made into another species. Finally, no control experiments were reported on blood or serum from the same individuals yielding the cerebro-spinal fluid. What hemodynamic actions are produced by human serum concentrated twenty-five times and injected into the vein of a rabbit?

All of these objections could be met by using the normal cerebro-spinal fluid of the same species and controlling its action with that of the serum or blood from the same animal. This report is a repetition of the experiments of Cushing and Goetch along this line. The following points were considered:

1. The hemodynamic action of the cerebro-spinal fluid.
2. The influence of the cerebro-spinal fluid upon sugar metabolism.
3. The influence of a Ringer's solution extract of the fresh hypophysis on sugar metabolism.

METHODS.

Collection of the cerebro-spinal fluid. — At first we planned to collect fluid enough from dogs under anesthesia by means of a fistula in the roof of the fourth ventricle, but it soon occurred to us that the fluid thus collected would not be sufficiently normal. Diminishing the hydrostatic pressure in the ventricles increases the rate of secretion of the fluid, while the rate of secretion of the hypophysis probably remains unaltered. If the hypophysis secretion passes primarily into the cerebro-spinal fluid, the fluid secreted under the above conditions would be more dilute than the normal. For that reason it became necessary to collect the fluid from ten to fifteen dogs, for each experiment. When the cerebro-spinal fluid is drawn from the fourth ventricle after the dog is bled to death, an 8 to 12 kilo dog will yield on the average 10 c.c. of the fluid. The fluid was collected under as nearly sterile conditions as possible, kept on ice, and used within one to two hours after being collected.

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[6] HERRING: Quarterly journal of physiology, 1911, iv, p. 183.

In most of the experiments upon the supposed hemodynamic action of the fluid, a quantity of blood equal to the fluid to be injected was withdrawn before the injection was made, in order not to change the quantity of fluid in the vascular system. We aimed to inject several times the quantity of cerebro-spinal fluid secreted during twenty-four hours, basing our estimation on the figures given by Halliburton for man.[7] The dogs used for experimentation on the sugar tolerance were not bled prior to the injection of the fluid or the hypophysis extract. Ether anesthesia was used in all the blood-pressure experiments.

THE ALLEGED HEMODYNAMIC ACTION OF THE CEREBROSPINAL FLUID.

The results are summarized in Table I, and two typical tracings are reproduced in Figs. 1 and 2. The hemodynamic action of the cerebro-spinal fluid appears to be due solely to the quantity of fluid injected, as is the case with the defibrinated blood or Ringer's solution. There is no pressor or depressor action, at least within one hour after the intravenous injection, one hour being the longest time that any of the experiments were continued. It seems that the quantity of the cerebro-spinal fluid injected, 50–100 c.c. into dogs averaging 3.5 to 7 kilos, was sufficient to produce distinct effects, if the hemodynamic substance is present in demonstrable quantities in the normal fluid. In view of our results the slight pressor action obtained by Cushing and Goetch may be due to their using foreign, pathological, and con-

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[7] HALLIBURTON: Biochemistry of muscle and nerve, London, 1904, p. 71.

TABLE I.

The relative Hemodynamic Action of Intravenous Injections of Dog's Cerebro-spinal Fluid, Defibrinated Blood, and Ringer's Solution into Dogs. Blood Pressure recorded from the Carotid Artery.

Dog.	Weight.		Blood drawn.	C. sp. fl. injected.	Def. blood injected.	Ringer's solution injected.	Blood pressure in mm. Hg.						
							Normal.	After bleeding.	During injection.	After injection.	Summary.		
											Normal.	End of injec'n.	Permanent.
	k.		c.c.	c.c.	c.c.	c.c.							
I . .	7.0	*a*	100	100	..	..	130	80	80 to 120	110	130	120	110
		b	100	..	..	100	130	70	70 to 120	110	110	120	110
II . .	3.5	*a*	50	50	..	..	160	130	130 to 170	150	160	170	150
		b	50	50	..	..	150	140	134 to 140	140	150	140	140
III . .	4.0	*a*	50	50	..	..	100	70	70 to 110	100	100	110	100
		b	50	..	50	..	100	65	65 to 110	90	100	110	90
		c	..	..	50	..	90	...	90 to 110	90	90	110	90
IV . .	4.5	*a*	75	75	..	..	150	130	130 to 150	140	150	150	160
		b	..	60	..	..	140	...	140 to 160	146	140	160	145
V . .	4.5	*a*	75	75	..	..	114	100	100 to 120	100	114	120	100
		b	..	..	..	75	100	...	100 to 120	110	100	120	110
		c	75	..	75	..	110	90	90 to 110	100	110	120	100
VI . .	4.8	*a*	75	75	..	..	130	120	120 to 130	130	130	130	130
		b	75	..	75	..	130	100	100 to 140	130	130	140	130

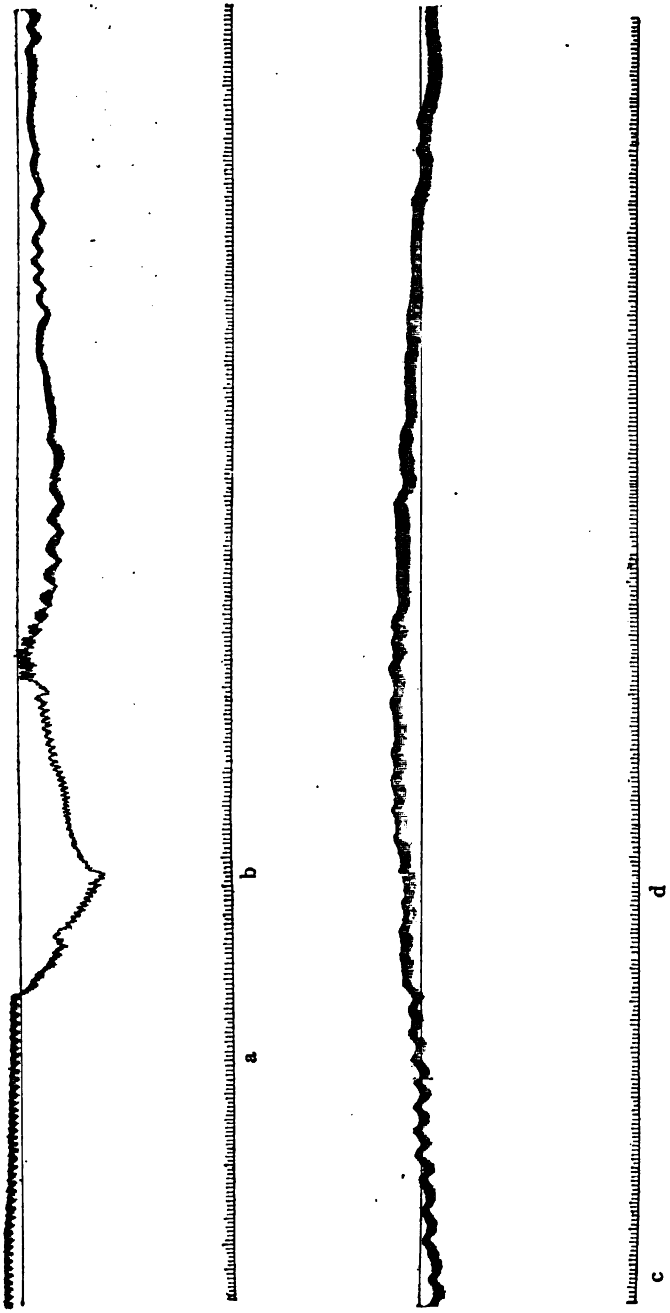

FIGURE 1. — Record of carotid blood pressure of Dog V. Weight, 4.5 K. *a–b*, withdrawal of 75 c.c. of blood from the femoral vein: *c–d*, injection of 75 c.c. dog's cerebro-spinal fluid into the femoral vein. Showing no distinct hemodynamic action of the c. sp. fluid. Time, seconds.

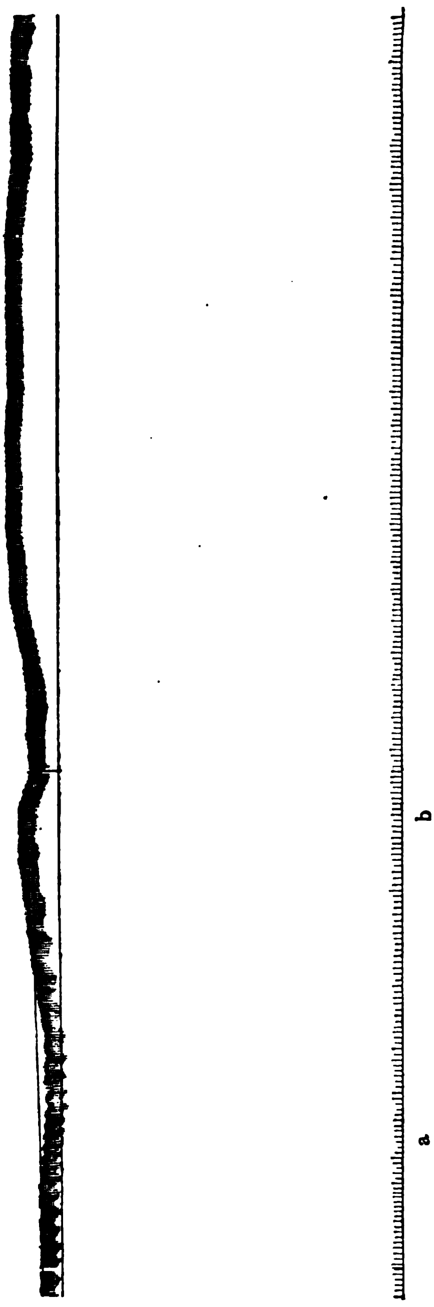

FIGURE 2 — Record of carotid blood pressure of Dog IV. Weight, 4.5 K. *a–b*, injection of 60 c.c. dog's cerebro-spinal fluid into femoral vein, without previous bleeding. Showing the temporary rise in pressure usually following the injection of an equal quantity of defibrinated blood, serum, or Ringer's solution. Time, seconds.

centrated cerebro-spinal fluid. Possibly their using rabbits for the tests is also a factor.

THE INFLUENCE OF INTRAVENOUS INJECTION OF NORMAL CEREBRO-SPINAL FLUID ON THE SUGAR METABOLISM.

The work of Borchardt,[8] and especially that of Cushing[9] and his pupils, seems to show that experimental hypopituitarism (posterior lobe) in dogs leads to an increased sugar tolerance, and that injection of the posterior lobe has an opposite action, and may even cause hyperglycemia and glycosuria. These results articulate with the frequent occurrence of glycosuria in acromegaly and gigantism, conditions that are supposed by some to be causally related to hyperpituitarism. In their latest work Cushing and his pupils ascribe this rôle in carbohydrate metabolism solely to the posterior lobe, and state that the secretion passes primarily into the cerebro-spinal fluid. If these two hypotheses are correct, intravenous injection of normal cerebro-spinal fluid ought to decrease the sugar tolerance or produce hyperglycemia and glycosuria in meat-fed dogs.

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[8] BORCHARDT: Zeitschrift für klinische Medizin, 1908, lxvi, p. 332.

[9] GOETCH, CUSHING and JACOBSON: Bulletin of Johns Hopkins Hospital, 1911, xxii, p. 165.

In all of our experiments a 20 per cent solution of glucose was used, made fresh, filtered and boiled. Thus the dilution was such that 5 c.c. of solution represented 1 gm. of glucose. In our preliminary experiments for determining the tolerance on intravenous injection we found the average was between 0.9 and 1.5 gm. per kilo body weight. This varied with different animals and even in the same individual from one week to another. There appeared to be a slight increase in tolerance upon repeated injections (one injection per day) of sugar. In our experiments we aimed to inject a quantity of sugar equal to or a little above the assimilation limit. The tolerance for each animal was carefully established before the experiment was started. The dogs were kept in metabolism cages, and particular care was taken to keep the urine free from contamination by food or feces. In most cases the urine was tested for sugar as soon as it was voided. Where Fehling's test was questionable, fermentation tests were made. All the sugar above the assimilation limit introduced intravenously appears to be eliminated within the first twenty-four hours following the injection. In most of our experiments, however, two or more days were allowed to intervene between successive experiments upon the same animal. The dogs used were kept on the ordinary laboratory diet of a mixture of cooked meat and bread.

Our results are summarized in Table II. They are clearly negative. Intravenous injection of large quantities of normal cerebro-spinal fluid has no effect upon the carbohydrate metabolism in the dog. This being the case, we should not expect to produce hyperglycemia and glycosuria on injecting the fluid into normal dogs receiving no sugar. But no experiments were made on this point, principally because of the amount of work involved in the collection of the requisite amount of cerebro-spinal fluid.

TABLE II.

SUMMARY OF EXPERIMENTS ON THE EFFECTS OF INTRAVENOUS INJECTION OF NORMAL CEREBRO-SPINAL FLUID (DOG) ON THE ASSIMILATION OF GLUCOSE INTRODUCED INTRAVENOUSLY IN THE DOG.

Dog.	Weight.	Date.	Intravenous injection.		Glucose in urine.
			C.-sp. fl.	Glucose.	
	k.		c.c.	gm.	gm.
I	8.2	April 18	. .	10	1.28
		" 20	40	9	0.07
		" 21	. .	9	0.07
		" 22	40	9	Trace
		" 25	. .	9	Trace
		" 26	23	9	0.07
		May 4	70	9	0.04
II	4.9	" 9	48	5	Trace
		" 11	. .	5	0.05
		" 15	65	5	. . . [1]
III	5.3	" 16	. .	5	0.1
		" 19	75	5	Trace
		" 22	. .	5	Trace
		" 24	75 NaCl	5	Trace
		" 25	. .	5	Trace
IV.	3.0	" 20	. .	3	0.07
		" 22	. .	3	0.04
		" 24	50 NaCl	3	Trace
		" 25	. .	3	Trace
		" 26	50	3	Trace
		" 30	. .	3	No sugar
	3.5	June 1	. .	3.5	Trace
V	4.0	" 2	60	5	0.4
		" 7	. .	5	0.35
VI	4.0	" 3	95	6	1.8
		" 6	. .	6	1.5

[1] Dog died during the night.

THE INFLUENCE OF INTRAVENOUS INJECTION OF EXTRACT OF FRESH HYPOPHYSIS (DOG) ON THE CARBOHYDRATE METABOLISM IN THE DOG.

In their extensive work of partial and complete hypophysectomy in dogs, Cushing and his co-workers frequently obtained glycosuria or lowered sugar tolerance lasting for one or two days after the operation. Cushing interprets this as due to the "squeezing out of the reserve deposit of posterior lobe secretion into the cerebro-spinal fluid, or into the wound, and its subsequent absorption into the blood."[10] This is an extension of the view held by many surgeons, in the case of the thyroid gland in exophthalmic goitre, the manipulation of the operation in exophthalmic goitre being so directed as to minimize the discharge of the thyroid secretion into the blood or wound. As regards the temporary glycosuria, and lowered sugar tolerance, in the pituitary operation, this condition may be due to a number of causes; for example, the injury to the peduncles and the base of the brain, and the anesthesia. But Cushing's hypothesis can readily be put to the experimental test. For if the mere handling or squeezing of the posterior lobe discharges enough secretion to cause glycosuria, then the introduction of one or more dogs' hypophyses into an otherwise normal dog ought likewise to produce glycosuria. If the posterior lobe secretion is in such a state in the gland that it can be forced into the cerebro-spinal fluid or into the wound by the mere handling of the gland, the secretion ought certainly to be present in a Ringer's solution extract of the macerated fresh gland. Most of our experiments were made on fresh glands, ground, extracted and injected immediately after removal from the animal, as it was not our purpose to go into the hemodynamic or general toxic action of tissue extracts or of the hypophysis in particular. The urine was tested for sugar for twenty-four to forty-eight hours after the injection.

Our experiments are summarized in Table III. The results are negative except in one case of injection of ten anterior lobes, when a trace of sugar appeared in the urine. Every experiment on the posterior lobe gave negative results, even when as many as ten lobes were injected at one time. The negative results are surprising, in view of the facts that the extracts of even a few dogs' hypophyses produce marked toxic symptoms such as vomiting, purgation, cardiac and respiratory irregularities, and lastly general depression. In view of these results, we are inclined to the view that the temporary glycosuria following operation on the hypophysis is due to the manipulation of or injury to the brain or to the anesthesia and not to any excess hypophysis secretion accidentally introduced into the blood.

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[10] GOETCH, CUSHING, and JACOBSON: *Loc. cit.*

TABLE III.

SUMMARY OF EXPERIMENTS ON THE EFFECTS OF THE INTRAVENOUS INJECTION OF THE EXTRACTS OF THE FRESH HYPOPHYSIS (DOG) ON THE CARBOHYDRATE METABOLISM IN THE DOG.

Dog.	Date.	Number of glands injected.				Remarks.
		Entire gland.	Posterior lobe.	Anterior lobe.	Sugar in urine.	
I . . .	June 11	3	..	..	None	Vomiting, heart irregular slow.
II . . .	21	10	..	..	None	Vomiting and purgation.
	23	12	..	..	None	Vomiting and purgation.
	24	..	10	..	None	Heart stopped: artificial resp., heart massage.
	27	..	5	..	..	Dog died in five minutes.
III . .	24	7	..	..	None	Vomiting.
	25	..	..	10	Trace	Purgation and vomiting.
	27	..	5	..	None	Purgation and vomiting.
	29	..	2	..	None	Purgation and vomiting.
	30	..	..	2	None	
	July 5	..	..	10	None	..
IV . .	June 28	10	..	..	None	
	29	..	10	..	None	..
	30	..	..	2	None	
	July 5	..	..	5	None	..
V . . .	5	..	10	..	None	
VI . .	5	..	5	..	None	..

The objection might be made to the above experiment that the hypophysis secretion may not be dissolved in the Ringer's solution, or it may be an unstable compound and hence destroyed in the maceration of the glands. In order to meet these criticisms we devised the following experiment. The hypophysis (entire gland) was extirpated aseptically from dogs immediately after being bled to death, and the glands (from one to three) were imbedded in the thyroid of a dog under light ether anesthesia. The hypophyses thus transplanted into the thyroid gland of a normal dog are entirely absorbed within forty-eight hours after the operation. This ought to insure a correspondingly rapid absorption of the hypophysis secretion and consequent hyperpituitarism. The results are as follows:

I. July 22. One dog's hypophysis transplanted into thyroid of a normal, 9 kilo dog. Urine of dog examined July 23–24: *No sugar.*

II. July 23. Three dogs' hypophyses transplanted into thyroid of a normal 10 kilo dog. July 24: *a trace of sugar in urine.* July 25: *no sugar in urine.*

III. July 24. Three dogs' hypophyses transplanted into thyroid of a normal, 6 kilo dog. July 25–26: *No sugar in the urine.*

The trace of sugar in the urine of Dog II on the day after the implantation of the pituitary glands can be accounted for by the ether anesthesia and the violent struggling of the dog on being handled and anesthetized. In Experiment III special care was taken to select a gentle dog, and of operating very rapidly and under light anesthesia. Although three fresh pituitary glands were introduced, the urine remained free from sugar.

CONCLUSIONS.

1. Normal cerebro-spinal fluid (dog) does not contain a pressor substance in demonstrable quantities.

2. Intravenous injection of normal cerebro-spinal fluid (dog) does not affect the sugar tolerance in the dog.

3. The intravenous injection of extracts of fresh pituitary gland, the posterior or the anterior lobes, or the implantation of the entire gland does not produce glycosuria in the dog. This is at least true for the quantity represented by from two to ten glands (dog).

These negative results do not prove that the cerebro-spinal fluid is free from hypophysis secretion. As a matter of fact, we have as yet no adequate test for the hypophysis secretion in the body fluids. When such tests shall have been worked out, we expect that the distribution of this secretion in the body fluids will prove to be similar to that of all the other internal secretions so far studied, the concentration being greatest in the blood and least in the cerebro-spinal fluid.

THE INFLUENCE OF ILLUMINATION OF THE EYE ON VISUAL ACUITY.

BY PERCY W. COBB.

I. INTRODUCTORY AND HISTORICAL.

THE fact that a bright light in the field of vision reduces the sensibility of the eye is one long familiar. The universal use of hats so constructed as to shade the eyes from the sky, and the unconscious interposition of the hand or some other object between the eyes and a bright light are bits of silent testimony as to this. It needs nothing further to show that this state of the visual field is at least uncomfortable. Very simple experiments are adequate to show that the visibility of objects may be distinctly less when a bright light is indirectly incident on the eye. On the other hand, under certain conditions it has been noted that side-illumination of the eyes has caused increase in the power of the eyes to see detail. This was pointed out by Sewall,[1] who showed that lines ruled on a card were visible at a greater distance when light from the sky was thrown into both eyes from the side than when all light was cut off from the eyes (except, of course, that coming from the test-card) by an appropriately constructed mask. Sewall explained this on the basis of a color effect brought about by the reddish light transmitted by the sclera, and overlooked the fact that pupillary contraction was also brought about by this side-illumination and with it a diminution of the effect of the refractive error of the eyes.

Urbantschitsch[2] found, in the case of his own eyes, that cutting off the vision of a test-object from one eye (without darkening that eye) caused a diminution in visibility of the letters on a test-card. Then,

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[1] SEWALL: Journal of physiology, 1884, v, p. 132.

[2] URBANTSCHITSCH: Archiv fur die gesammte Physiologie, 1883, xxxi, p. 280.

(1) darkening the unused eye caused vision to become still less acute, usually after an interval of about ten seconds, but (2) at times this effect was reversed. Darkening the unused eye then caused an immediate and usually transient increase of visibility for detail and uncovering the eye again caused a diminution. The effect (1) he considers cannot be due to pupillary dilatation, because, first, it is too tardy (pupillary dilatation is complete in one second), and, second, certain experiments on pathological eyes, where vision was reduced by cloudiness of the media or impairment of the light-sensitive organs, showed corresponding changes in *color visibility* where the pupil of the eye experimented upon had lost its power of response to light.

As to the case of a light source in the field of vision of the tested eye, Urbantschitsch found vision for details increased when his own eye was exposed, and less again when the eye was screened from the light. In cases of complete pupillary inactivity and paralysis of accommodation he found that there was, when the eye was exposed to the direct light, an apparent *brightening* of the test-object, and *vice versa*. In this case he says absolutely nothing about acuity of vision for details, presumably because in these eyes vision was too much reduced. He also overlooks the purely physical fact that the scattered light in the eye from the direct rays of the light source could easily account for the apparent brightening and darkening of the test object, especially where pupillary compensation is shut out. This instance then does not show heightening of retinal sensibility in any respect, due to a light source in the field of vision.

Schmidt-Rimpler[3] used a side-illumination of the eye transmitted through the sclera. By a lens system, the image of an electric lamp filament was thrown on the exposed sclera of the eye and vision for details investigated under this condition. He found that, using a side-illumination of low intensity, he could get an increase of visibility, and with higher side-illumination a decrease. This first effect was also obtained in atropinized eyes, from which he comes to the conclusion that the side-illumination may bring about a heightening of visual acuity independent of pupillary contraction. Later Uhthoff[4] and Depène,[5] using similar methods, worked out the influence of an excentric light-source on central vision, the latter very completely, with the following results:

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[3] Schmidt-Rimpler: Verhandlungen der XIX ophthalmologischen Gesellschaft zu Heidelberg, 1887, p. 76.

[4] Uhthoff: Sitzungsbericht des IX internationalen ophthalmologischen Congress, Utrecht, 1899.

(1) When the test-object is under sufficient illumination to give vision = 1.25 or over (on Nieden's system), the side-light was capable of bringing about an increase in visual acuity.

(2) At lower illuminations on the test-object, an excentric light source caused a diminution in visual acuity which was greater, (*a*) the lower the illumination on the test-object, (*b*) the smaller the angle at the eye made by the side-light with the line of direct vision, (*c*) the greater the glare ("Blendung"), (*d*) the greater the retinal area stimulated by the excentric light.

This last effect (diminution of visual acuity) Depène ascribes to retinal adaptive changes. The first effect (1) he concludes is due to changes in the pupillary opening brought about by the incidence of the side-light. Where mydriatics or myotics were used to effect a constant pupillary diameter no increase in visual power was observed under these conditions except in one case, where a slight pupillary mobility could also be detected.

It was also found by Depène that where the eye was shaded so that the side-light fell upon the pupil only, a wholly similar set of results was obtained. If the sclera alone transmitted the light from the side-source, similar results were obtained, but the changes with change of angle of incidence of the side-light were less marked or absent.

Borschke[6] investigated the question, and came to the conclusion that the disturbance of vision with bright light incident on the eye was wholly due to a physical cause, namely, the imperfect transparency of the media transmitting the light which caused a certain amount of scattered light to fall on the retina, chiefly near the image of the light source, which acted as a veil over the image of the visual test-object. He concludes that the fibrillary structure of the lens is the chief cause of this scattering, mentioning however several other less significant factors. These are reflection from the retina, entrance of light into the eye through the sclera, particles of mucus, etc., on the corneal surface and the floating particles in the air.

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[5] Depène: Klinische Monatsblatt für Augenheilkunde, 1900, xxxviii, p. 289 and p. 390.

[6] Borschke: Zeitschrift für Psychologie und Physiologie der Sinnesorgane, 1904, xxxiv, p 1.

Borschke[7] used a test-object illuminated by transmitted light, the source for this purpose being mounted on a track and approximated to the test-object until the latter was just legible. At this point the distance from lamp to test-object was called M. When the glare-light (a circle of six lamps arranged about the test-object with a diffusing medium before them) was thrown on, it was found that the first lamp had to be moved nearer the test-object to make it just legible. This distance was called N, and M/N was the measure of the disturbance of vision by the glare.

In twelve subjects, of ages from twelve to sixty years, using one eye alone, corrected with proper glasses (− 5.5 D to + 2.75 D in the extreme cases), although M varied from 12 to 1.5, the value M/N was approximately constant, varying from 1.9 to 2.5. Further,[7] with a focal source for the glare-light, he found that when this was 90° from the line of fixation, it was immaterial whether the light was on the nasal side, and therefore throwing its retinal image outside the sensitive retina, or on the temporal side, where its image fell within the sensitive area; also, that the progressive decrease of vision with approximation of the glare-light to the line of fixation was wholly uninterrupted as the image of the glare-light fell on the blind spot.

Lastly, placing the test-object so that its image fell on a fixed point of the retina, 15° away from the point of fixation, he found that the interference with vision increased progressively, not as the image of the glare-light approached the fovea, but as it approached the image of the test-object, 15° away from the fovea in the visual field.

These facts he thinks go to show that adaptive disturbance of the eye plays a very small part, if any, in this phenomenon, and that the chief factor is a physical one. At least in his work he states that he found no evidence which made it necessary to assume that an excentric light in the field of vision brought about a change in the retinal state on which the depression of visual acuity could depend.

II. THE PRESENT WORK.

1. **Apparatus and general considerations.** — A specially devised test-object was used in this work. When two systems of parallel lines

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[7] BORSCHKE: Zeitschrift für Psychologie und Physiologie der Sinnesorgane, 1904, xxxv, p. 161.

are superimposed, making a very small angle with each other, a set of bands appears in direction at right angles to the bisector of the acute angle. These bands are formed by the minute rhombi arranged side by side, representing the parts common to the spaces of both gratings, which are the bright elements of the system. By varying the angle between the two gratings the bands become broader and more widely separated as the angle is diminished and *vice versa*. For this idea the credit is due to Dr. H. E. Ives of this laboratory.

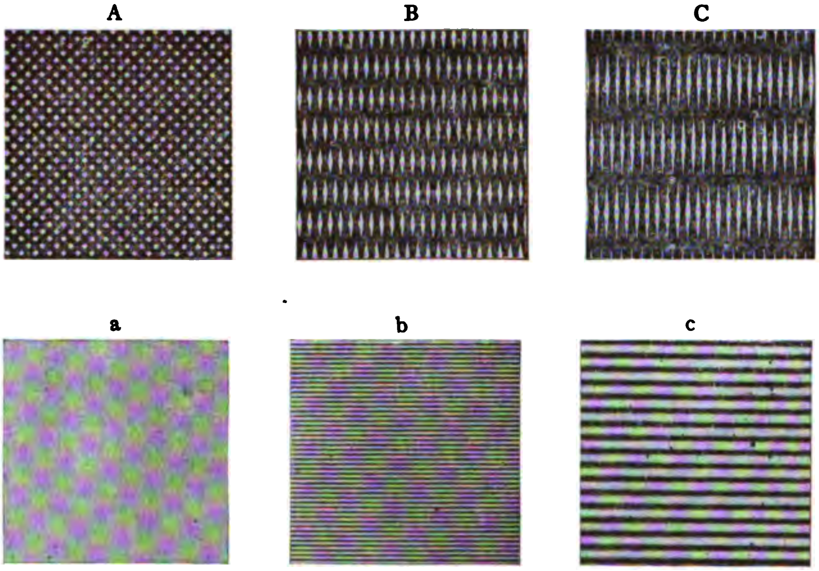

Figure 1.—*A*, *B*, and *C*, photomicrographs (× 17); *a*, *b* and *c*, appearance, actual size, of gratings in different positions. The bands in *B* and *C* become clear when viewed at a distance of three or four metres.

A glance at Fig. 1 will make the principle of this instrument clear. Three positions of the gratings are shown, as they appear microscopically and as viewed from a distance. The gratings used are of 240 black lines to the inch ruled on plate glass 1/8 inch thick, separated by clear lines of equal width, and are such as are used in photoengraving work. The mounting for the gratings was designed by the writer, and made by the mechanician of the laboratory, Mr. Wm. Würth. It consists of two rings, in which the gratings are mounted, so arranged as to turn freely on each other. The ends of the U-shaped piece (*a*, Fig. 2) bear one on a lug on one ring, the other on a lug opposite to this on the other ring, so that as the U is advanced by the micrometer screw the rings are rotated in opposite ways. This motion is opposed by a spiral spring (*b*) which is attached by its two ends to the two rings respectively so as to oppose the advance of the U. The micrometer reads to 1/50 mm.

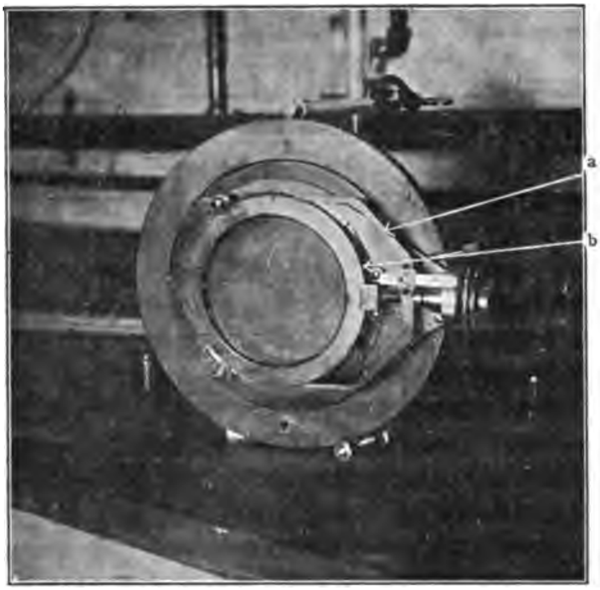

FIGURE 2.—The gratings and their mounting.

For a mathematical exposition of the principle involved in this instrument, the reader is referred to the published description of it.[8] Briefly, visual acuity at the point where the lines are just visible is exactly proportional (other conditions remaining the same) to the sine of half the angle made by the gratings with each other. Since the two limbs of the U act on the rings in a nearly tangential direction, and the angles used are quite small, the displacement of the U as measured by the micrometer is directly proportional to visual acuity with a very slight and negligible error. The micrometer is adjusted to read 0 when the gratings are exactly parallel to each other, and all of the values obtained in the present work are expressed in terms of the micrometer readings.

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[8] IVES: Electrical world, 1910, lv, p. 939.

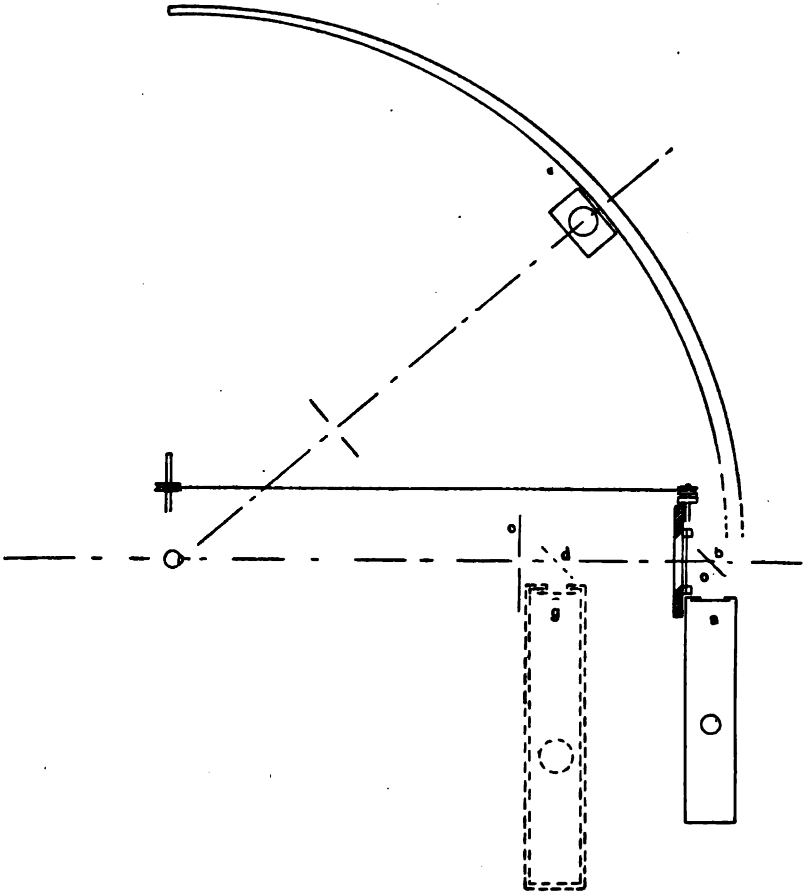

Figure 3. — General arrangement of apparatus.

It is clear that this instrument furnishes a test-object that can be indefinitely varied, from what appears to be a flat gray to a system of bands of any visible width up to the size of its field. This is accomplished, further, without any concomitant disturbing circumstance that alters either the intensity of illumination on the object, or that of any adjoining parts of the field of vision. The change is simply in the width of the lines and nothing else. In this way are avoided the disturbing features of changing from a smaller to a larger test-object, of changing the distance of the object from the eye, or of changing the illumination of the test-object. The subject can himself adjust the test-object to the limit of visibility by means of a cord running over the pulley on the micrometer head, and in the present work the writer was both subject and observer, the readings being taken through a telescope by a slight shift of the head with the eye which is *not* the subject of experiment. As a support for the side-light a Wundt perimeter was used (Fig. 3), the working radius of which is 1 metre. The test-object was placed at the fixation point (*o*), illuminated from behind by the reflection of a milk-glass surface (*a*) in a small mirror (*b*). By a screen (*c*) the size of field was cut down to 2° or to about the limit of the rod-free area of the retina.

More constant readings were obtained when a part of the field was magnified some 6 or 7 per cent. This was accomplished by parts of spectacle glasses fitted into a single frame, the central vertical, parallel-sided piece having a refractive power of 1 diopter, the left-hand segment of 2 diopters, the remaining (right-hand) segment being a plane glass. When placed before the test-object and 3½ cm. from it, as viewed from a distance of 1 metre, the parts of the field appear magnified (from right to left) 0, 3½, and 7 per cent, and the point at which the lines in the left field are visible, those in the right field invisible, and those in the middle field doubtful, was accepted as the limit of visibility.

It is to be said at once that this point under fixed conditions with all disturbing influences ruled out, and pupillary change as far as possible excluded by the use of a diaphragm of 2 mm. diameter close to the eye, was found to fluctuate in successive observations far and away beyond the limits that the precision of the apparatus itself would lead one to expect. The best series of forty readings taken in the course of fifty-five minutes with brief rests gave the following:

Average of first 10	7.49
" " second 10	7.48
" " third 10	7.54
" " fourth 10	7.58
General average	7.52
Largest single reading	7.83
Smallest " "	6.95
Maximum fluctuation	11.7 per cent of average.

It will be seen that, apart from irregular fluctuations from one observation to the next, this series exhibits a slight rise in the course of less than an hour. Further, variations of a larger order were observed from day to day under identical conditions. These may in part be due to practice, but outside of this for some unaccountable reason a variation of not less than four per cent (in the averages of ten) is frequently seen on successive days, or even in successive series of ten, in such an irregular way as to prohibit the supposition of fatigue or skill due to practice to account for the difference. Of ten series similar to the one cited taken at different times and averaged in groups of ten, the average increase from the first to the second group for all the series was (expressed algebraically) −0.012; from the second to the third, + 0.09; from the third to the fourth, − 0.03. The extreme differences between successive groups were both between the first and second, this range being from − 0.33 to + 0.18 (where the average of all differences was − 0.012). Although, then, in some cases the difference is quite large, it is irregular and inconsistent in sign, and does not on the whole show itself to have any definite relation to the time the eye was subject to the conditions of the experiment.

The foregoing were taken with a certain amount of general illumination in the room, namely, the light from a 60-watt (about 48 candles) tungsten lamp in a corner of the room (about 10 × 15 feet) somewhat back of the observer, the general illumination being sufficient to get about the room easily and to take notes, but insufficient for comfortable reading.

It was of interest to determine whether any change in the capacity of the eye to resolve detail took place by reason of prolonged stay in feeble light or in the dark. It was found that if the room was illuminated by the indirect light of an 8 c. p. lamp reflected from the wall (a fairly light yellowish tan color), with the test-light at 6 cm. distance from the milk-glass, that vision dropped from 7.49 (average of ten observations) to 7.24 in the course of two hours, a drop of 0.25, which is insignificant as compared with the differences just mentioned. At a lower illumination on the test-object (lamp at 40.6 cm.) a rise from 4.61 to 4.77 was observed, also of insignificant magnitude and in the opposite sense.

It can be concluded then that a difference of 4 or 5 per cent between two successive series of ten observations is without significance in the present work, and that there is no effect large enough to be comparable to this at the luminosities of test-object used in this work that could be ascribed to adaptation or an analogous process in the retina.

2. **Results of present work.** — A point of interest as to the reason of the reduction of vision by the incidence of a side-light on the eye is the question as to whether this is mainly physical, the result of the scattering of light by the media and other tissues of the eye, or whether it is due to the well-known reciprocal action of the retina, the induced physiological darkening of the retina in the neighborhood of a brightly illuminated part. In connection with this the behavior of the eye when the image of the side-light occupies the blind spot is interesting. Under these conditions careful observation is required to know whether the image of a light-source is on the blind spot or not. Using as a test-object a white spot on a black surface, seen by reflected light, the white spot wholly disappears when its image falls on the blind spot. When a light source is so used, the actual image of the source itself does disappear, but a bright glare is still visible. By shifting vision slightly away from the point of fixation, or by having the lamp moved slightly by an assistant, it can be seen to emerge from invisibility. By properly directing the attention, the lamp becomes visible, as though it were being advanced from behind a screen. At the same time, with the lamp wholly invisible there is enough light falling on the sensitive retina to make a brilliant, diffuse spot of light evident to the observer, by which he would be able unhesitatingly to locate the lamp.

A comparison was made to determine the amount of depression of vision for detail resulting (*a*) from the light whose image fell on the blind spot, (*b*) the same light swung into a meridian 15° higher up. For each position of the side-light twenty observations were taken, alternating with controls during which the eye was shaded from the side-light by a suitable shutter in this order: side-light off, on, on, off, off, etc. This alternation helped to eliminate the effect of the fluctuations in the capacity of the eye which have just been discussed. This was repeated for position *b*, then the whole repeated in the reverse order, that is, (1) *b*, (2) *a*. This made, controls and all, forty observations each for *a* and *b*. The whole was repeated for four different illuminations of the test-object. Before the eye was placed an artificial pupil with an opening 2 mm. in diameter, small enough in outside measurement so that the side-light could fall unobstructedly on the pupil of the eye. This was done so that pupillary fluctuation could not cause a variation in the amount of spherical aberration of the eye.

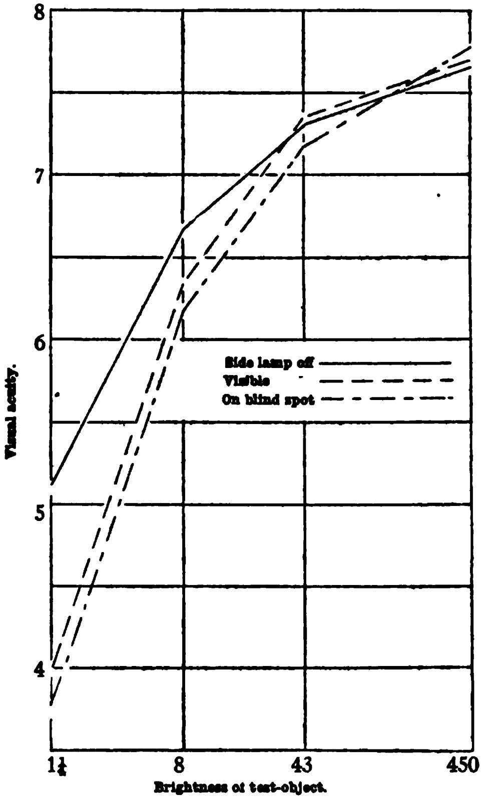

Figure 4.—Behavior of vision when the image of the side light falls on the blind spot.

For each test-object illumination the controls corresponding to the values for *a* and *b* were found to disagree slightly, as was to be expected. The two sets of controls were accordingly averaged, and the values for *a* and *b* so corrected as to bear the same proportion to this average as they did to their respective controls. The results are embodied in Fig. 4, where the abscissæ are drawn proportional to the logarithms of the brightness of the test-object. The crossing of the curves, from the fact that at the highest brightness of the test-object, vision was better with side-illumination of the eye than without it, is in agreement with previous observations of others. The smallness of the difference at that point, however, would make it doubtful in this case as to whether it was due to accidental variation or to actual increase in visual acuity.

It will be seen that for the lower luminosities of the test-object the blind-spot effect is constantly greater than when the sensitive retina is concerned. It is true that although here the variations in intensity of retinal illumination and optical accuracy of the eye as far as the test-object was concerned were eliminated by the artificial pupil, the variations in illumination as to the side-light were left at the mercy of the natural pupil. Assuming that this was slightly larger when the blind spot was the part of the retina chiefly illuminated, we would have a proportionately larger ratio of illuminations (side-light: test-object) of the retinal images than in the parallel case when the sensitive retina was concerned, hence a lower value for visual acuity, as is seen in the results.

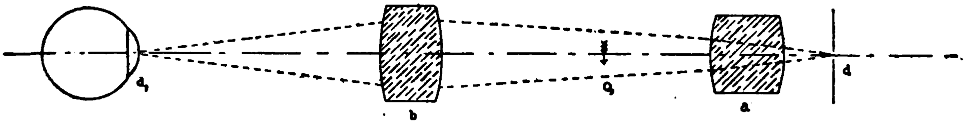

FIGURE 5.—Optical system used to secure the effect of an artificial pupil without obstructing side illumination of the eye.

On the other hand, the higher reflecting power of the nerve-head, as shown by its paler color, should, as Hering has pointed out,[9] cause a larger amount of light to be scattered within the eye when the image of the light source falls upon it, which would reduce vision by a correspondingly larger amount. Which of these two factors is chiefly concerned in the present instance remains undetermined. The fact, however, remains that, with due allowance for this circumstance, the reduction of visual acuity was not less when the blind spot was the seat of the side-light image than when this was placed the same distance from the centre on the sensitive retina.

To determine the relation between the depression of visual acuity and the angle of incidence of side-light on the eye, a generally similar method was used. To obviate the possibility of an artificial pupil partially shading the eye from the side-light an optical system was devised, shown in Fig. 5. With the test-object at *o*, not shown in the figure, the first lens *a* threw an image of the same at o_1, which was looked at through a second lens *b* so placed that the light rays left it parallel. The lens *a* was a focusing glass, found on measurement to have a focal length of 3.87 cm.; the lens *b* a 2-inch (focal length) Hastings triplet magnifier. Both lenses were achromatic combinations. A diaphragm with an opening 1.58 mm. diameter was placed at *d* so that its image formed by the combination of the two lenses fell at d_1, or in the pupil of the observing eye, and was about 2 mm. diameter. In this way it was assured that the illumination of the retinal image of the test-object was dependent on the size of the diaphragm d, whose image at d_1 was small enough (2 mm., more or less) to fall wholly within the natural pupil and hence to exclude the influence of its variations. The natural pupil under the conditions of experiment was found to have a diameter of 5 mm. or over. By slightly shifting a, the spherical error of the eye could be compensated, and by a suitable cylindrical lens between o_1 and b the astigmatic error. This last correction was in a measure adjustable, using the same cylinder at different distances from o_1. This arrangement caused a certain amount of distortion, in that the circular field of the test-object was elongated horizontally by the convex cylindrical lens placed at the point described with its axis vertical, but the vertical dimension which involved the width of the lines of the test-object was not sensibly altered. The distance o to d was 80.5 cm. The opening equivalent to d (as far as light transmission is concerned) at 1 metre was then $\frac{1.58 \times 100}{80.5} = 1.96$ mm. The glass parts of the eye piece were found to reduce the luminosity of the image by absorption to 69.4 per cent. The lamp illuminating the test-object was 20 cm. from the milk-glass. This absorption was equivalent to placing it at 24 cm.

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[9] HERING: Grundzüge der Lehre vom Lichtsinn, Leipzig, 1907, in Handbuch der gesammten Augenheilkunde, 2 Auflage.

The readings were taken (for each position of the side-light) as follows: a series of ten successive readings with the side-light screened off from the eye, then a series alternating exactly as in the preceding experiment, of twenty readings, ten with the side-light on the eye and ten with the side-light screened off. Four such series of thirty observations were taken for each position, (1) with a ground glass before the lamp, (2) with three layers of clear glass (found to give nearly the same illumination on the eye) in place of the ground glass. The four series were taken in the order (1), (2), (2), (1), never more than two a day and always several hours apart. The results are given in Table I, where each value is the average of twenty readings. In the curve (Fig. 6) the average of all of the dark readings (initial and alternate) is put as 100 for each angular position of the side-light, and the average of the readings with the side-light on the eye shows a progressive loss of visual acuity as the side-light approaches the visual axis. The curve then shows the mean of the values for clear glass and ground glass as stated in Table I.

It is natural to assume that the dark readings taken alternately with the readings for side-illumination of the eye should be the ones used as a basis of comparison. However, when so computed with the dark readings put as 100, in each case, the curves for ground glass and clear glass over the side-light are irregular. The curves obtained putting the result of the initial dark series at 100 are consistent with each other. The average of the two curves for ground glass and clear glass is fairly smooth in both cases, and shows a distinct increase in the rate at which visual acuity decreases with angle of side-illumination at a point about 25° from the visual axis, not essentially different from Fig. 6.

TABLE I.

RELATION OF VISUAL ACUITY TO THE ANGLE OF INCIDENCE OF THE SIDE-LIGHT, AND TO THE NATURE OF THE LIGHT SOURCE.

Angle of incidence of side-light.	Clear glass.			Ground glass.		
	Initial controls.	Alternate controls.	Side-light on.	Initial controls.	Alternate controls.	Side-light on.
90	5.75	5.68	5.62	5.93	5.78	5.69
60	5.72	5.67	5.41	5.83	5.82	5.47
40	5.92	6.01	5.52	6.00	5.89	5.54
25	6.08	5.93	5.51	6.15	6.14	5.54
12	6.05	5.91	5.10	6.08	5.90	5.01

In each case, too, it was found that on the whole there was a slightly greater depression of vision where the ground glass was used than where the image of the filament was visible, contrary to the result anticipated. As in preceding results relating to the blind spot, the retinal image of the side-light was dependent as to luminosity on the width of the pupil of the eye, while the image of the test-object was controlled by an artificial pupil, so that changes in the pupil with changing conditions would introduce a disproportion between the two. The fact that the light-source in the two cases caused stimulation of a different extent of retinal area (although the total light falling on the retina was, *cæteris paribus*, the same) offers an explanation for a possible difference in pupillary diameter in the two cases. The pupil was not measured.

The following series was planned to show how far the assumption that light scattered from the eye media and tissues could be held accountable for the reduction in visual acuity brought about by a light-source in the field of vision. Series were made comparing (*a*) the reduction in vision due to side-illumination of various intensities with (*b*) the reduction in vision due to a veil or haze thrown over the retinal image of the test-object. In the first case (*a*) a side-light (100-candle stereopticon lamp) was placed on the perimeter arc at an angle of 32½° outward in the horizontal meridian. This was covered with a black felt cloth, fastened over a framework, forming an improvised lantern, of which the side toward the eye was a cardboard screen with a suitable hole in it. In front of this an adjustable sector disc was rotated. The full intensity of the light could be obtained with the disc stationary. The largest opening gave two open sectors, each of 90°, which in rotating the disc cut the light down to one half. The disc was graduated so that by shifting the two parts on each other the intensity of the light could be cut down in stages as follows: 1, ½, ¼, ⅛, $\frac{1}{16}$, $\frac{1}{32}$ of the whole intensity.

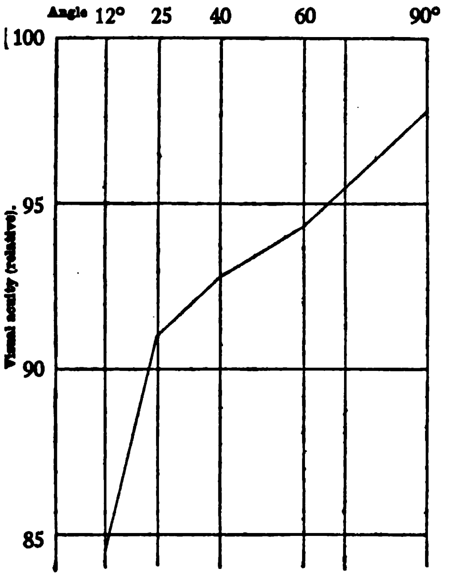

Figure 6.—Relation between angle of incidence of side-light and resulting depression of visual acuity.

To shut out possibility of pupillary fluctuation a double artificial pupil was used, made of thin brass, with two openings 2 mm. diameter and bent so that the side-light passed normally through one opening and fell on the pupil while the test-object was viewed through the other. A small amount of practice made it possible to see by indirect vision that the side-light was well in view through its opening.

This set of observations was compared with (*b*) a second set obtained by interposing an oblique plane glass (*d*, Fig. 3) in the line of vision, in which by partial reflection the image of a second glass (*g*) was visible to the eye at the same time that the test-object was visible through the oblique glass reflector. The outlines of these were made to coincide by the margins of the opening in the screen (*c*) in the line of vision. The brightness of the "haze" so thrown over the test-object was so adjusted by a lamp mounted on a suitable track as to be definite fractions and multiples of the average brightness of the test-object, namely, ¼, ½, 1, 2, 4, 8, as measured by a photometer. At each setting, either (*a*) or (*b*), three readings were taken at each intensity of side-illumination, and at each value of haze-brightness after the first three with vision uninfluenced, going always from the lower values to the higher. Five (in one case six) such series, taken not oftener than two a day, gave the values cited. Each value is then the average of 15 or 18 individual readings. Two intensities of illumination of the test-object were used, the haze-brightness just mentioned being referred to the higher test-object brightness as unity. The results are given in Table II.

TABLE II.

COMPARATIVE EFFECT ON VISUAL ACUITY OF SIDE-ILLUMINATION OF THE EYE AND HAZE.

	RELATIVE INTENSITY OF SIDE-ILLUMINATION.						
Brightness of test-object.	0	1/32	1/16	1/8	1/4	1/2	1
4.85	6.78	6.53	6.41	6.21	5.90	5.46	4.90
1.21	4.86	4.48	4.12	3.87	3.37	2.65	2.08
	BRIGHTNESS OF HAZE = 4.85 MULTIPLIED BY						
	0	1/4	1/2	1	2	4	8
4.85	6.65	6.47	6.33	6.14	5.81	5.25	4.68
1.21	5.11	4.87	4.52	4.24	3.61	2.84	1.88

In order that the two sets of values should start from an identical zero value, where conditions were identical, the corresponding zero values were averaged, and the other values all corrected so as to bear the same proportion to this average as they did to their respective zero values. By plotting the curves of the values so corrected, using logarithms of the intensities of side-illumination or haze-brightness as abscissæ, we can superimpose the curves and slide one on the other horizontally without destroying the proportionality of these values. This procedure shows at once (Fig. 7) that the diminution of vision for haze followed a different law from that for side-illumination, in the sense that for haze the diminution in vision was always more rapid with proportionate increase than in the case of simple side-illumination of the eye.

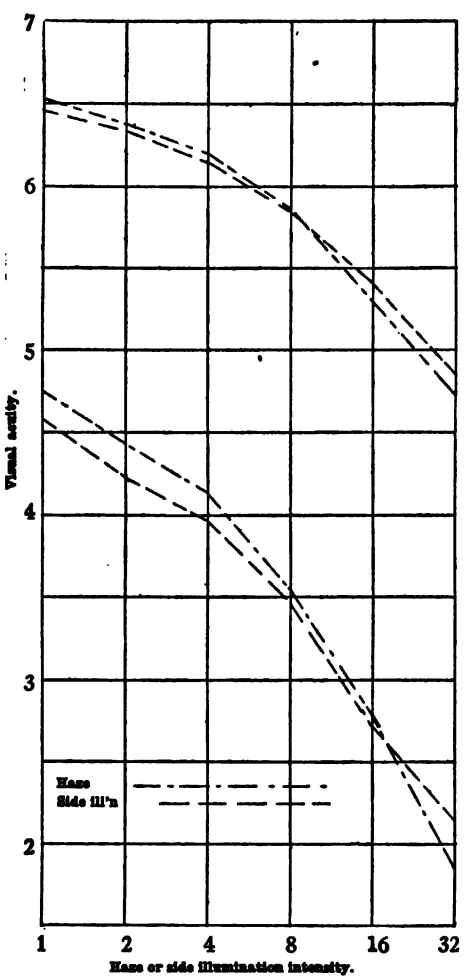

Figure 7. — Graphic representation of values given in Table II.

III. Discussion.

It is of interest here to take some figures from Depène's work[10] and deduce from them a few quantitative relations of the various factors in the problem. The curves in Fig. 8 give his visual acuity values for an angle of incidence of the side-light of 10° (except the lower curve as noted). The abscissæ are proportional to the side-light intensity; that is, to the squares of the diameters of diaphragms placed before a gas mantle which was the actual source, the diaphragm opening being the virtual source of light. Although not directly stated, from figures given in another place it is inferred that with full opening (20 mm. diameter) the intensity of side-illumination on the eye was 53.6 Normal-Kerzen (48 metre-candles). With this value before us we can pick out points on the curves where the illumination from the side-light on the eye is equal to the intensity of illumination on the test-object (*e*, *e*, etc.). At a glance it is evident, then, that this condition involves no diminution of vision worthy of consideration.

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[10] Depène: *Loc. cit.*

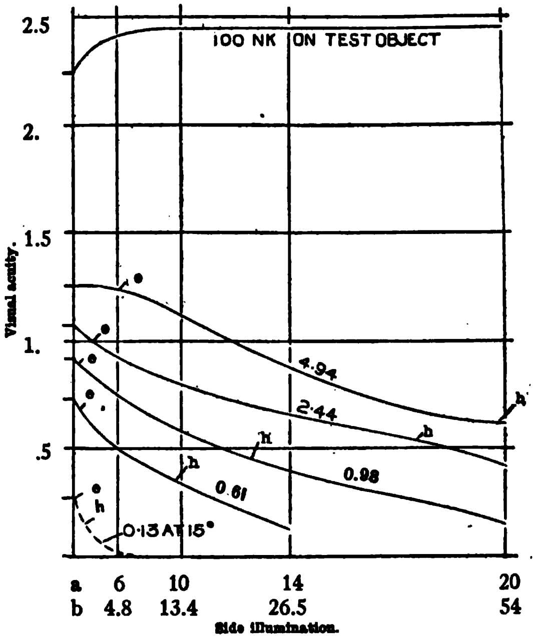

FIGURE 8. — Behavior of vision with side-illumination at 10°, from Depène's data. The abscissæ are (*a*) diameter in millimetres of diaphragm which acted as side-light source; (*b*) resulting illumination in Normal-Kerzen on the eye from the side-light.

It is found that when the illumination on the test-object is such that protected vision is 1.25 Nieden's system (about one half its maximal value) the illumination on the eye must be about 11 times that on the test-object to reduce vision to one half its protected value. At intensities of illumination on the test-object less than this the ratio is about 20 (points *h*, *h*, etc.). These observations were carried on at illuminations as low as 0.13 Normal-Kerzen, which gave protected vision the value 0.33.

In simple terms this means that a lamp equally distant from the eye and the test-object (black characters printed on white paper in this case) cannot materially reduce vision of detail provided the lamp is not less than 10° from the visual axis. If the lamp is moved toward the eye (or the eye toward the lamp) until the test-object is three to five times as far from the lamp as the eye is, a considerable loss of visual power will result. These considerations are not shown to hold as far as the highest illumination (100 Normal-Kerzen) on the test-object is concerned, since the maximum side-illumination used on the eye barely exceeded one half of this.

The hypothesis that the reduction of visual acuity from side-illumination of the eye is due to the presence of scattered light over the retinal image of the test-object is shown by the curves in Fig. 7 to be wanting, since, although the curves are similar, they represent different functions. There is no constant proportionality between the values of side-illumination and haze-brightness which will produce identical disturbances in the values for visual acuity. We may however estimate such a proportionality which shall hold approximately over a limited range. By shifting the curves horizontally on each other (which is allowable, since the abscissæ are drawn logarithmically) they can be brought to an approximate coincidence in certain parts, and the proportion (such as mentioned) that exists estimated as it applies to those parts only. The proportions so obtained are given in Table III. It is to be remembered that these values are wholly relative. The figures under 2 represent the high values for side-illumination and haze, approaching 145 metre-candles in the case of side-illumination and a haze equal in brightness to a magnesium carbonate block with an illumination of 31 or 32 metre candles upon it. Under 1 the values 145 and 50 or 56 represent the proportion only, the values actually considered being from 1/8 to 1/32 of these.

TABLE III.

Haze Values (expressed as Metre-Candles on a Magnesium Carbonate Block, making it appear equally bright) equivalent in the Disturbance of Visual Acuity Values to Side-Illumination of the Eye of 145 Metre-Candles coming from a Light Source 32½° from the Visual Axis.

Brightness of test-object.	1 Upper stretch of curves.	2 Lower stretch of curves.
4.8	50.0	32.3
1.2	56.1	31.2

The figures show what has already been graphically shown by the curves, the divergence between the results of side-illumination and haze for different proportional intensities.

The average value from Table III is 42.4. This would mean, if a constant value had been obtained, that the average illumination on the fovea due to scattered light from a light-source at 32½° from the visual axis is the same as the illumination on the fovea when the eye is looking directly at a magnesium carbonate block illuminated by the same lamp removed from the block $\sqrt{\frac{145}{42.4}} = 1.85$ times as far as the lamp was from the eye in the first place. For the lower intensities this value is $\sqrt{\frac{145}{53}} = 1.65$; and $\sqrt{\frac{145}{31.7}} = 2.14$ for the higher.

These figures give an idea (a very rough one) of the order of magnitude of the scattered light on the fovea for side-illumination of the eye, while at the same time they show that the interference with vision does not by any means depend wholly on scattered light over the image. If it did we should certainly get a closer correspondence of the two sets of curves just discussed. There must then be another effect, a change in sensibility of the part of the retina concerned, brought about by the light which enters the eye from the side-light source, and falls elsewhere than upon the image of the test-object. This change in the retinal state may, for brevity's sake, be called induction.

There are then three possible factors we can consider as the cause of the diminution of vision by side-illumination of the eye:

(1) The image of the side-light source itself on the retina which may reduce the general retinal sensibility by induction.

(2) That portion of the scattered light from the same which superimposes itself upon the image of the test-object and so reduces the contrast in the latter.

(3) The scattered light falling upon the remainder of the retina, and especially that contiguous and near to the image of the test-object, which influences the state of the portion of the retina upon which that image falls by induction.

As to the first of these, the image *per se* of the side-light, it was shown by Borschke[7] that when this fell on the temporal margin of the retina (the light in the visual field at 90° nasal) the interference with vision was the same as when it fell on the opposite margin at the same angle, although in the first instance the light source itself was invisible. Further, the same observer found that when the image of the side-light fell on the blind spot the effect did not diminish, a point which the present work confirms. Lastly, Borschke, viewing the test-object by excentric vision, found that the magnitude of the interference with vision increased with proximity of the side-light image to that of the test-object, independently of the part of the retina on which the side-light image fell. That is, the effect was the same whether the side-light was viewed foveally or by the peripheral retina at an equal angle beyond the test-object in the visual field.

It is true, Heymans[11] found that vision was unaffected when the second stimulus fell on the blind spot. He, however, used the threshold value as a measure of vision, and apparently worked at a much lower order of intensities of stimuli, so that his work is hardly relevant to the present discussion.

It may fairly be concluded, the writer believes, that the image of the side-light source, by itself, is an almost negligible factor in the resulting reduction of vision.

As to the second factor, the scattered light falling upon the image of the test-object, the conclusion can hardly be escaped that this plays an important part. Yet the facts that side-illumination and haze give different sets of results and that no constant proportional relation can be found between them, leads to the conclusion that something is added to this in the case of side-illumination, relatively more active at the lower intensities. There seems but one more factor to consider, and that is the scattered light on the retina, not on, but close to the image of the test-object, which works by "induction," that is, by reducing the general sensibility of the retina in that region and so reducing the visibility of the test-object. If, as there is reason to believe, the image of the side-light itself on the retina is a negligible factor in the resulting depression of vision and the effect of scattered light falling *directly upon* the retinal image (which alone is considered under the name of "haze" in this work) increases more rapidly with increase of intensity than the effect of side-illumination, as a whole, as has just been shown, it must be concluded that the effect of scattered light falling on the retina *about* the image of the test-object must increase more slowly with increase of intensity than the effect of side-illumination as a whole. This conclusion remains to be confirmed by subsequent work.

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[11] Heymans: Zeitschrift für Psychologie und Physiologie der Sinnesorgane, 1901, xxvi, pp. 333 ff.

While, then, it is concluded that the reduction of visual capacity from side-illumination is due practically altogether to light scattered upon the retina, and that the image of the light-source upon the retina in itself is a negligible factor, we cannot extend these conclusions to cover the phenomena of discomfort and possibly fatigue included in the meaning of the word "glare." Indeed it can be stated with confidence that visual discomfort on the one hand and the lessening of the actual capacity of the eye to see detail on the other, are two separate and distinct effects of side-illumination.

In the series mentioned, where the effect of side-illumination (1) with ground glass interposed and (2) with the lamp filament visible, were compared, the intensity of light falling on the eye was very nearly equal in the two cases. Yet, although the ground glass (1) caused a slightly greater depression of visual function, there was in the case of the visible filament (2) a distinctly greater sense of discomfort. Changing from this to the ground glass gave the impression of passing out of a noisy place into a quiet one, a distinctly soothing effect. A similar result is a matter of more or less common experience, an electric lamp well out in the field of vision may, if the bulb is a clear one, cause distinct irritation, and its replacement by a frosted lamp of even higher candle-power will give distinct relief.

Borschke found that vision in one eye was scarcely affected by placing a bright light before the other, and has come to this conclusion: "dass das unangenehme Gefühl der Blendung und die durch Blendung hervorgerufene Sehstörung volkommen verschiedene Begriffe sind, und keineswegs gleichzeitig in gleichem Grade vorhanden sein müssen." [12]

It was thought that possibly the fluctuation of individual observations of the same series in the present work might go hand-in-hand with this discomfort. Accordingly from the separate series involving the use of ground glass as compared with the visible filament, in each run of ten observations the mean deviation from the average was calculated, and these for the whole experiment when averaged showed almost identically the same fluctuation in the case of ground glass as in the case of the visible filament.

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[12] Borschke: *Loc. cit.*, xxxv, p. 172.

On the whole, then, the actual reduction in the visual power does not explain the discomfort resulting from a light source in the field of vision. This must be sought in other directions, and it is possible that an examination of the eye movements under these conditions may open the way to a more complete understanding of the question.

CONCLUSIONS.

(1) Fluctuations in the limit of capacity of the eye to perceive detail occur within a few minutes, or from day to day. These are not to be explained by inexactness of the method of measurement.

(2) In the averages of successively taken groups of ten observations, variations of 4 or 5 per cent frequently occur, in a way not to be explained by either fatigue or the effect of practice.

(3) Conclusions from variations in visual acuity within this range can only be drawn (*a*) when observations are alternated with controls or (*b*) when series of observations are repeated to a point of agreement.

(4) On the whole, no systematic change was observed in the present work, which could be attributed to change in sensibility of the eye during the time within which the series was taken. The maximum time was fifty-five minutes.

(5) Prolonged stay in feeble light caused no noteworthy change in visual acuity.

(6) Light from a bright source entering the eye reduces the visibility of an object the more, the brighter the source; the lower the brightness of the object of vision, the smaller the angle at the eye subtended by the two, (except that where the test-object is very bright, illumination of the eye has been noted to increase visibility).

(7) Under a condition imitating the worst practical condition for reading (the light at 10° from the visual axis, equal illumination of the eye and the test-object, in this case printed characters on white paper) the reduction in visual acuity is negligible at any intensity of illuminations.

(8) The retinal image of the light source in itself is a negligible factor in the depression of vision, at least at angles of 15° and over,

since, other conditions being equal, it is indifferent whether the image falls on blind or sensitive portions of the retina.

(9) The depression of vision is due to light, which, by reason of reflection or diffusion, partly from imperfect transparency of the eye media, is scattered over the retina upon and near the image of the object of vision.

(10) Visual acuity behaves in general, but not wholly, the same with (*a*) illumination of the eye from a source and (*b*) a haze of light thrown over the rod-free central retina (subtending 2° in the visual field) with proportional variations in the light-flux in the two cases.

(11) The disagreement between the two sets of results just mentioned must be due to changes in the sensibility of the part of the retina concerned in vision of the object, induced by the scattered light in the case of illumination of the eye, falling on the retina not on, but about that part, and probably not farther away from it than 15° measured in the visual field (the remoteness of the blind spot).

(12) There is no parallelism between the depression of vision for detail on the one hand, and discomfort and other visual disturbances classed under the head of "glare" on the other hand, resulting from a light source in the field of vision. What is stated in this paper has only to do with the quantitative relations of visual acuity. The genesis of these other disturbances is to be sought in other directions.

THE REACTION OF THE VASOMOTOR CENTRE TO ASPHYXIA.

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INTRODUCTION.

AT the meeting of the Society for Pharmacology and Experimental Therapeutics at Boston in December, 1909 (Journal of pharmacology and experimental therapeutics, vol. 1, p. 571), we presented briefly a method for studying the reactions of the vasomotor centre, and the preliminary results obtained in this way. In the interim we have extended these investigations and amplified and confirmed the results, of some of which we now wish to give the essential details.

The method consists essentially in the artificial perfusion of an organ which is completely severed from the natural circulation, by cutting or ligating all its blood vessels, but which is left in connection with the vasomotor centre of the animal. In this way the drugs, etc., cannot act upon the vessels directly, so that peripheral actions are excluded, as also variations of blood supply from cardiac disturbance, etc. The details of the method and the results obtained with sciatic stimulation and with curare have been described by us in this journal, 1910, xxvi, pp. 233–259.

1. **The response to complete cessation of respiration.** — This was produced in curarized dogs, maintained alive by the continuous insufflation of oxygen through a small catheter passed deep into the trachea. The experiment consisted simply in interrupting the stream of oxygen. The use of curare excluded any muscular complications.

With the vasomotor centre in good condition (as shown by fair initial blood pressure and rise on asphyxia, Experiments C 1, 11, 12, 15, 71), there is progressive and very marked slowing of the spleen flow (Fig.

1), starting during the rise of blood pressure, growing much more pronounced as the blood pressure falls, and usually reaching its maximum just after the heart has stopped. Evidently, therefore, the *fall* of blood pressure in asphyxia is not due to central vasomotor paralysis, but must be purely cardiac, although the blood pressure excursions (even of the membrane manometer) may appear exaggerated. On the contrary, the centre becomes the more active as the pressure falls and remains so for a brief time after the heart has stopped. When the cardiac arrest is sudden, the vasomotor stimulation may be altogether post-mortum. When the asphyxia is relatively slow, or the vasomotor centre partly exhausted, the stimulation may pass off before the heart is quite arrested, as in Fig. 1.

The stimulation of asphyxia is stronger than that producible by any other means. In the experiments quoted, the rate of flow was decreased to one half to one tenth of the original; the average flow was about 22 per cent of the original. The results are the same whether the vagi are intact or divided.

2. **When the vasomotor centre has been paralyzed,** there is naturally no slowing of flow with asphyxia, as in the following experiments:

Experiment 18. — The centre had been destroyed by repeated hemorrhage and did not react to sciatic stimulation, although the blood pressure was brought back to 55 mm. by the injection of the blood and the infusion of normal saline solution, 40 c.c. per kg.

In Experiment 19, shock was induced by handling, cooling, and crushing the intestine. The pressure was thus reduced gradually to 10 mm., but there was still a rhythmic though feeble pulse when the oxygen was turned off.

3. **When the blood pressure is low** (about 40 mm.) **but the vasomotor centre intact,** asphyxia may cause a marked slowing of the flow (reducing it to 40 to 65 per cent), although the blood pressure does not rise — presumably because the heart cannot respond to the vasoconstriction (Fig. 2) (Experiments 14 and 29).

4. *Cardiac arrest* seems to occur more promptly, when the level of blood pressure is at or below 80 mm., when the oxygen is withdrawn.

In five experiments, with blood pressure of 90–120 mm., death occurred in five to eight and one-half minutes; average, six and one-third minutes.

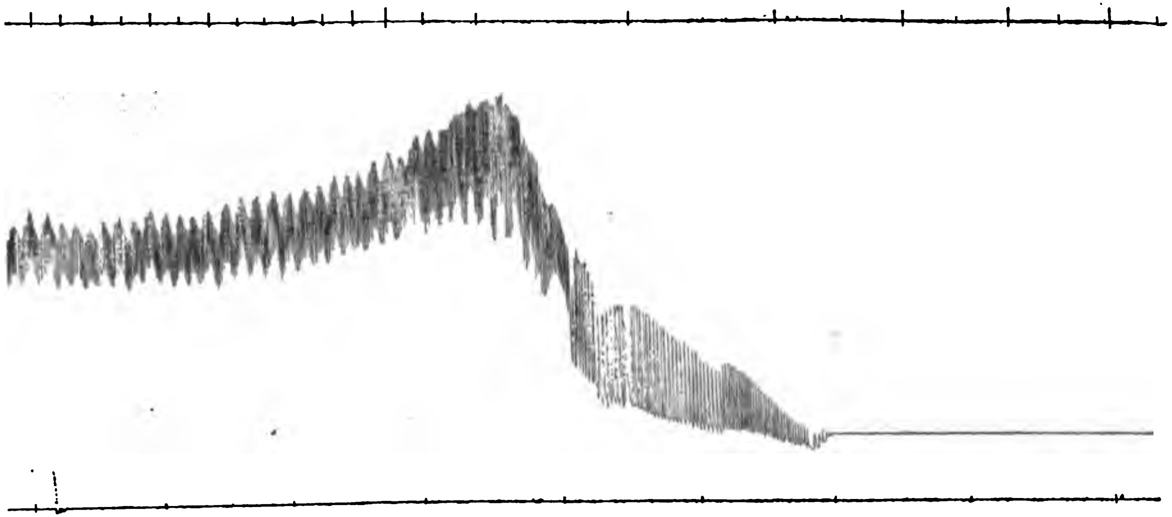

FIGURE 1. — *Typical effects of asphyxia on blood pressure and vasomotor centre.* The upper line shows the rate of vein flow from the perfused spleen. 'Note that the slowing (corresponding to vasoconstriction) reaches its maximum *after* the pressure falls. (Experiment C, 12-2. Dog, curare, oxygen insufflation, vagi intact.)

In three experiments, with blood pressure of 55–80 mm., death occurred in three to four minutes; average, three and two-thirds minutes.

In three experiments, with blood pressure of 40 more or less, death occurred in two and one-half to five minutes; average four minutes.

5. **Asphyxia by sudden arrest of the heart.** — The intravenous injection of overdoses of strophanthus (corresponding to 5 mg. of the drug per kilogram of animal — Experiments C 20, 47, and 63) causes a marked rise of blood pressure during which the heart becomes suddenly arrested, about two to five minutes after the injection. During the rise the spleen flow is commonly quickened, but as the heart stops, a very powerful slowing sets in, strictly comparable to that seen in ordinary asphyxia but even more pronounced. In the three experiments quoted, the flow was reduced to 12 to 36 per cent, average 18.5 per cent of the original flow. The same phenomenon is seen when the heart is arrested by other means:

Clamping of thoracic aorta (*Experiment 42*). The pressure rose from 80 to 100 mm., with some quickening of the flow. When the clamp was released after one minute, the pressure fell rapidly, the heart weakened and stopped in one minute. Concomitant with this fall, the flow was also slowed progressively till death.

Overdosage of epinephrin (*Experiment 49*). The injection of 1/20 mg. of epinephrin per kg. raised the pressure from 160 to 260 mm., which was quickly followed by arrest of the heart. Immediately after this cardiac arrest and fall of blood pressure, the spleen flow was markedly slowed.

6. **Recovery of the flow after death.** — In sudden death as by strophanthus, the extreme constriction may persist up to five minutes (Experiments 20 and 47). In asphyxia, by stoppage of oxygen, the constriction begins to lessen usually within one minute. The averages of seven representative experiments (C 1, 12, 15, 29, 41, 49, 80) are as follows: If the rate of flow before asphyxia is taken as 100, the rate at the time of the maximal slowing was 26 per cent, one to two minutes after death, 81 per cent, three to five minutes, 95 per cent. In four experiments, in which there was but little slowing at death (C 2, 13, 17, and 32), the flow was practically unchanged to five minutes after death.

The post-mortum paralysis of the vasomotor centre, and the consequent loss of tone, should lead to an eventual quickening of the flow.

We found this in five of sixteen experiments, in which the rate of outflow became from one fourth to two and one-half times faster than that before the asphyxia (Fig. 2). In the other eleven experiments the flow did not quite recover its former rate (Fig. 1).

In about one fourth to one hour after death, the flow again begins to be slower, and this slowing tends to increase over the two and a half hours covered by our observations. In most of the experiments this post-mortum slowing did not equal the asphyxial slowing.

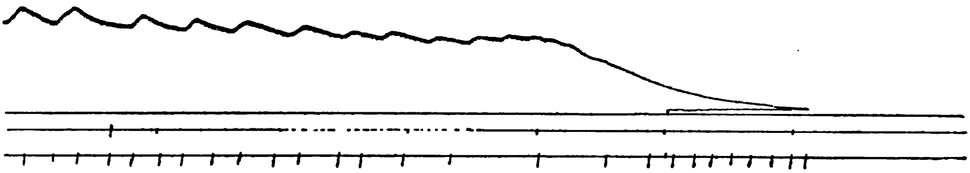

FIGURE 2. — *Asphyxial slowing of flow (lowest line) without rise of blood pressure.* The bottom line shows the rate of vein flow from the perfused spleen. This occurs when the blood pressure is at or near "shock level," before the asphyxia is started. It does not correspond to true shock, in which the slowing would be absent. (Experiment C, 14–2. Curarized dog, oxygen insufflation, spleen flow, vagi intact. The blood pressure had fallen spontaneously and slowly from 70 to 45 mm.)

7. **Asphyxia by clamping the trachea in non-curarized animals.** — This causes the same slowing during the fall of pressure and subsequent paralytic quickening (*e. g.*, Experiment 109), although the presence of convulsions often makes the results unreliable.

8. **Partial asphyxia and recovery.** — When the asphyxia is interrupted before actual death results, the vasomotor centre returns towards its original tone. That is, if the respiration is restored during the asphyxial vasomotor stimulation, the flow again becomes more rapid (*f*, *i*, Experiments 17, 27, 63); if the vasomotor depression has set in, the rate again becomes slower (*f*, *i*, Experiment 109). This return toward the normal state sets in almost immediately, but there remains generally a moderate increase of vasomotor tône which persists at least over ten minutes (Fig. 3).

When the interruption of the respiration is very brief (one fourth to one minute) the restoration of the previous tone is almost immediate and complete, so that this may be used for testing the excitability of the vasomotor mechanism of the animal (*f*, *i*, Experiments 128, 2: 109, 3).

9. **Partial asphyxia by breathing into a long tube.**— This was used occasionally to test the vasomotor mechanism of non-curarized animals, by attaching a piece of "gas tubing" about four feet long to the tracheal cannula for one or two minutes. In sensitive animals this suffices to give a moderate or even a very marked slowing (*e. g.* 78 *B*), sometimes more noticeable than the rise of blood pressure; but in some other animals the results were not as satisfactory.

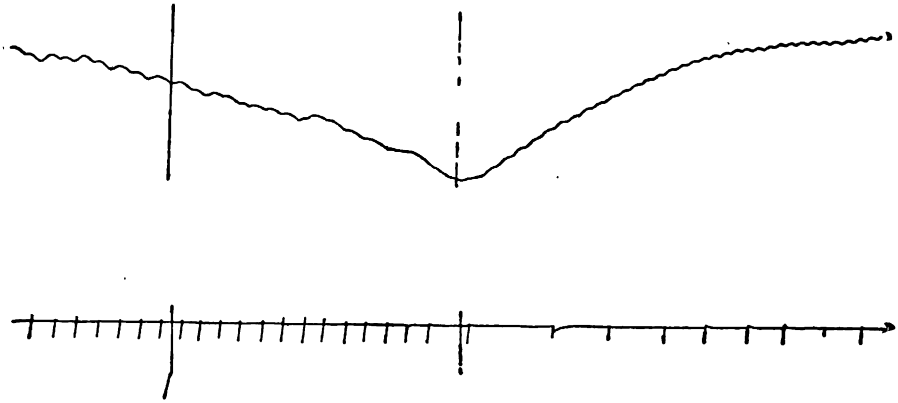

FIGURE 3. — *Partial asphyxia and recovery.* (Experiment 17-3. Curarized dog, spleen perfusion, vagi intact. Lowest line shows the rate of vein flow.) The stream of oxygen had become too slow, with the production of the asphyxial slowing. The stream is quickened and the slowing is thereby almost immediately lessened.

10. **Very gradual asphyxia.** — Experiment 75, in which the trachea was partially obstructed by mucus, furnished a good example of gradually developing asphyxia, with progressive stimulation of the vasomotor centre. It is also interesting as showing that the kidneys participate in the asphyxial vasoconstriction:

Experiment 75, dog; natural respiration, section of both vagi.

Minute after division of vagi.	0	5	10	15	20	25
Blood pressure . . .	105	145	150	140	145	140
Flow	100	80	55	42	22	40

11. **Partial asphyxia by pneumothorax.** — Puncture of the thorax (for the purpose of inserting a finger to compress the aorta) generally slows the flow, even when oxygen is being insufflated. That this

slowing is simply asphyxial is shown by the fact that it may be obviated by increasing the flow of oxygen (Experiments 46 and 41).

12. **Carbon monoxid asphyxia.** — Our method is well suited to determining whether the asphyxial vasomotor stimulation is due to excess of carbon dioxid or to deficiency of oxygen. We have not yet investigated the effects of carbon dioxid directly. To note the influence of exclusion of oxygen, we replaced the insufflation of oxygen in curarized animals, by a stream of ordinary coal gas of about equal speed of flow. In this way an anoxhemia would be produced rapidly, whilst the carbon dioxid would be removed at the same rate as before. The blood pressure in these cases falls without primary rise, but *the flow is not changed* (Experiments 17 and 27, Fig. 4).

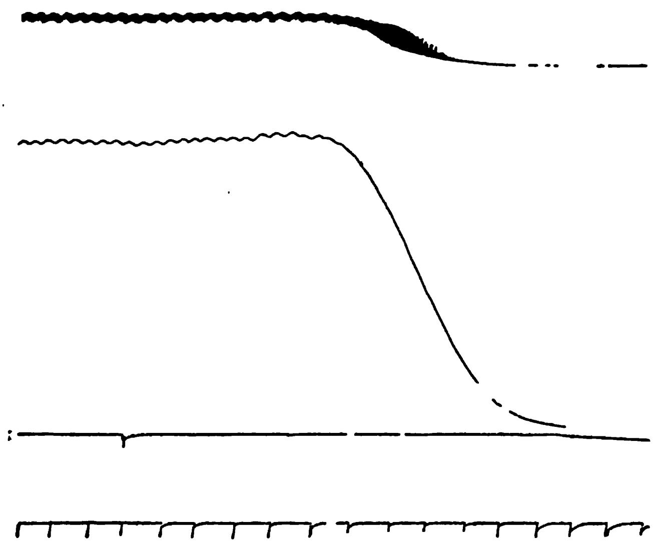

FIGURE 4. — *Carbon monoxid asphyxia.* Lowest line the rate of vein flow. Note the absence of slowing. (This tracing was taken about ten minutes after Fig. 3, from the same animal. The high blood pressure (130 mm.) shows that the vasomotor centre is intact. The uppermost tracing was taken with a membrane manometer.)

The time of death is about the same as with simple stoppage of oxygen, or perhaps somewhat more rapid (three animals, blood pressure 100 to 130 mm., died in three to eight minutes, average five minutes. (With simple stoppage of oxygen the average time of death, with the corresponding blood pressure, was six and one-third minutes.)

In two other experiments, a stream of coal gas was passed by catheter into the trachea of non-curarized dogs, with spontaneous respiration. In one of these (54) the results were as described — fall of blood pressure and nò slowing (or rather, slight quickening of the flow). In the other (56), the pressure fell steadily during ten minutes from 170 to 80 mm., with the flow practically unchanged. At this point the natural respiration began to fail, becoming slowed; the stream of gas was evidently not sufficiently rapid to remove the carbon dioxid without the aid of the respiration movement; for now the pressure rose to 120, with slowing of the flow, and then fell rapidly to death with the typical, severe asphyxial slowing.

It may therefore be concluded that ***asphyxial vasoconstriction is not due to deprivation of oxygen,*** but is caused presumably by the accumulation of carbon dioxid.

13. Whilst the results so far described have been mainly on the spleen, the conclusions may also be applied to the kidney, for the prepared kidney responds to asphyxia by the same slowing (Experiments 38,75, 79, 81 B, and 121).

CONCLUSIONS.

Asphyxia causes a marked stimulation of the vasomotor centre, which becomes extremely powerful as the blood pressure falls, and which may persist for a few minutes after death.

Analogous results are produced by sudden arrest of the heart.

The stimulation is not due to deprivation of oxygen and does not occur if the accumulation of carbon dioxid is prevented.

PALMESTHETIC DIFFERENCE SENSIBILITY FOR RATE.

BY KNIGHT DUNLAP.

[*From the Psychological Laboratory of the Johns Hopkins University.*]

NOTWITHSTANDING the numerous clinical observations on palmesthetic sensibility,[1] no attention has been given, so far as I am aware, to the question of difference sensibility for rate in this sensory realm. Can two different rates of vibration be discriminated through the "vibration feeling" alone? If discrimination is possible, what are the smallest perceptible differences for rates within the limits of the musical scale? Not only are these questions intrinsically interesting, but they also have some importance for the theories of acoustic sensibility.

Having become interested in the problems of palmesthetic difference sensibility primarily because of the support which their solution might conceivably bring to the "telephone" theory of audition, I began some experiments bearing on these problems, and my first results seem to have enough importance to warrant their brief presentation in print.

I tuned a set of eleven small forks (originally all of the "Philharmonic" a_1) to form a series ranging in rate from four hundred and twenty to four hundred and sixty vibrations per second by steps of four vibrations. I had the stems of the forks threaded, and had three small discs turned out of brass, each disc having a hollow stem at right angles to its centre, with female thread so that it could be attached to any of the fork stems. When one of the forks (with disc attached) was set in vibration by striking it on a piece of wood, and the disc was pressed on the skin of the hand, the vibrations were distinctly felt. Any two or three of the eleven forks could be used in a series of tests.

---

[1] The term "palmesthesia" and its cognates are derived from the Greek *palmos*, a quivering, or vibration. The term was introduced by RYDEL and SEIFFER (Archiv für Psychiatrie und Nervenkrankheiten, 1903, xxxvii, p. 488), but rendered by them "Pallästhesie," through an evident misderivation. On the nature of the sensory process, see, in especial, GOLDSCHEIDER, 1904, Berliner klinische Wochenschrift, 1904, xli, pp. 353 *et seq.*

In each test the subject placed his hand (or, in some cases, both hands), palm upward, upon a piece of canton flannel folded to form a cushion nearly an inch thick and lying on a table. In some cases the subject was blindfolded. In all other cases an opaque screen was interposed between the eyes and hands of the subject.

The general method of the first experiments was to strike two forks and then apply them (that is, to apply the discs attached to the stems of the forks) in succession to the same area, or as nearly as possible the same area on the hand. Each fork was applied for a period of time ranging from one to two seconds, and the interval between the removal of the first fork and the application of the second ranged from one-half second to one second. Both forks were struck before either was applied, and while one was being applied the other was held close to it (about an inch away, but not touching the skin). Sometimes the forks were struck with approximately equal intensity; sometimes one was struck harder than the other. Sometimes the one struck first was the first applied; sometimes it was the second. By taking these precautions it seemed possible to rule out the influence of auditory stimulation through air conduction, and also the influence of intensity differences in the palmesthetic stimulations.

In only a few cases were the vibrations audible to the subject. With my ear rather close (six inches) to the skin surface to which the fork was applied, I could notice a slight change in the sound when the disc was brought in contact with the skin or removed from it. Several of the subjects whom I tested by applying the fork to my own hand, held near the subject's ear, noticed this change in the sound; but none of them were able to discriminate between the change due to applying the fork and the change due to removing it.

To exclude the possibility of a "subconscious" influence of auditory stimulation through the air, and to exclude also the hypothesis of influence through bone conduction, further experiments were undertaken, in which two forks were simultaneously applied, one to each hand. I may here remark, however, that I had no idea that the two factors mentioned actually entered, as the forks were light, and gave a strong palmesthetic sensation, but gave a very faint auditory sensation, even when held a short distance from the ear. Heavier forks, which are suitable for auditory experiments on account of the stronger auditory sensations they produce, I found to give a much weaker palmesthetic sensation.

In most of the tests in which the forks were successively applied, the subject was required to judge if the vibration rate of the second fork of

THE RESULTS OF THE TESTS.

Subj.	Set.	Date.	Type.	Forks.	Pairs.	Right.	Per cent.
R. E. B.	1	April 18	A, o.	420, 460	..	..	...
	2	19	A, o.	420, 460	80	76	95
	3	19	A, o.	420, 440	80	75	93.75
	4	20	B, o.	420, 440, 460	100	83	83
	5	23	B, o.	420, 436, 452	100	90	90
	6	23	A, n-o.	420, 436	100	99	99
	7	23	A, n-o.	424, 432	93	..	...
	8	24	A, o.	424, 436	100	73	73
	9	24	A, o.	420, 444	100	99	99
	10	25	A, n-o.	420, 436	67	..	..
	11	25	A, n-o.	420, 444	100	95	95
	12	May 29	C, o.	420, 452	53	50	94.3
	13	29	C, o.	420, 444	53	50	94.3
	14	30	C, o.	420, 436	100	75	75
	15	30	C, n-o.	420, 436	100	81	81
C. W.	1	April 27	A, o.	420, 460	50	50	100
	2	27	A, n-o.	420, 452	50	47	94
	3	27	A, n-o.	428, 452	50	49	98
	4	May 2	C, o.	428, 456	100	71	71
S. W.	..	April 27	A, n-o.	428, 452	50	45	90
J. B. W.	..	27	A, n-o.	428, 452	50	49	98
M. W.	..	27	A, n-o.	428, 452	50	49	98
C. E.	1	May 24	A, o.	420, 456	113	101	89.3
	2	24	A, o.	420, 436	100	86	86

a pair was higher than, lower than, or equal to the rate of the first fork. In other cases the subject was merely asked to look for a difference, other than in intensity, between the sensations aroused by the two forks. In each set of experiments there were as many pairs in which the higher

THE RESULTS OF THE TESTS—*Continued.*

Subj.	Set.	Date.	Type.	Forks.	Pairs.	Right.	Per cent.
J. R. G.		June 21	A, o.	420, 456	100	48	48
G. E. D.	1	May 2	A, o.	428, 456	50	43	86
	2	June 21	A, n-o.	420, 456	22	10	45.4
	3	21	A, o.	420, 456	100	74	74
	4	21	A, o.	428, 456	100	78	78
	5	21	A, o.	428, 436	250	176	70.4
	6	21	C, o.	420, 456	50	30	60
	7	22	A, n-o.	436, 456	100	62	62
	8	22	C, n-o.	436, 456	80	26	32.5
	9	22	C, o.	420, 456	100	78	78
	10	23	C, o.	420, 456	150	121	80.6
	11	23	C, n-o.	420, 456	50	39	78
	12	23	C, n-o.	432, 456	80	40	50
	13	23	C, n-o.	428, 456	80	70	87.5
	14	23	C, o.	424, 444	50	41	82
	15	23	C, n-o.	432, 456	50	26	52
K. D.	1	23	A, n-o.	428, 456	50	36	72
	2	26	A, o.	428, 456	50	38	76
	3	26	A, n-o	428, 456	100	82	82
	4	26	A, n-o.	432, 456	100	12	12
	5	27	A, n-o.	436, 456	50	12	26
	6	27	A, o.	436, 456	50	26	52
	7	27	A, o.	436, 456	50	50	100
	8	27	A, n-o.	436, 456	25	25	100

fork came first as there were pairs in which the higher came last, or, if there were an odd number of pairs in the set, there was but one pair more of one sort than of the other. The two sorts of pairs were in all cases given in an irregular order which was determined by chance.

In sets which are marked "A," only two forks were used. In sets marked "B," three forks were used, the one of mean pitch being always applied first, and being followed sometimes by the higher, sometimes by the lower.

The sets in which the forks were simultaneously applied are marked "C." In each of these sets only two forks were used, the higher being applied to the right hand as often as to the left, in irregular, chance-determined sequence. The subject was required to determine whether the "high" fork was on the right hand or on the left hand.

In some sets the subject was told after each judgment whether that judgment was right or wrong. These sets are marked "o" (orientation). In other sets the subject was given no information as to the accuracy of his judgments. These sets are marked "n-o" (no orientation). In most cases the association of one fork with the term "higher" and of the other with the term "lower" was entirely built up through sets of tests with orientation.

The skin area selected for the tests with all of the subjects except G. E. D. was on the ulnar side of the palm, at about one third of the distance from the wrist to the base of the little finger. The skin at this place was horny enough to pick up the vibrations strongly. With G. E. D., an area near the little finger was used. His sensations were stronger from this area than from areas nearer the wrist, and the skin appeared to be thicker in the selected area.

R. E. B. is a schoolgirl thirteen years old, of musical training. In the cases where several sets were taken in one day, at least ten minutes' rest was allowed between sets. Set 6 was taken four hours after set 5. In sets 7 and 10 the judgments were based entirely on intensity differences, the fork which was vibrating the more strongly being taken to be the lower. In set 6 a difference of twenty-four vibrations was judged correctly ninety-nine times in one hundred trials, but in set 10 the same difference was below the threshold.

C. W. is an undergraduate student, slightly musical. He at first thought the difference between two forks was one of intensity only. His judgments, however, did not show any influence of actual intensity differences. At the end of the tests he said that the "lower" fork was *rougher* than the "higher" one.

S. W. is an undergraduate, quite musical. He too thought the differences between the forks were intensity differences, but, like C. W., was not influenced by actual intensity differences. When the terminology *smooth* and *rough* was suggested to him, he thought these terms might express the differences very well. The set on this subject and the sets on J. B. W. and on M. W. are remarkable, inasmuch as these subjects were given no practice, and no information whatever as to the correctness of their judgments.

J. B. W. did not designate the forks "higher" and "lower," but used the terms "finer" and "coarser," calling the fork actually higher, "finer." I counted the consistency in the application of these terms as correctness of discrimination, for the use of any terms to indicate the directly sensed difference in the vibrations is a matter of convention.

M. W. observed the taking of the series on J. B. W., and in the series taken on her immediately following it, she used the same terms ("fine" and "coarse").

C. E. is a scientific man and an accomplished musician. J. R. G., a graduate student, is pronouncedly unmusical. Immediately after taking the set on J. R. G., I took some auditory sets on him, striking two forks and then placing them successively on a resonator. In forty such trials with forks 436 and 440, the subject gave nineteen correct judgments. In forty-five trials with forks 420 and 440, the subject gave thirty correct judgments, and in five cases judged the two forks to have the same pitch.

G. E. D. is an undergraduate student, musical. He noticed a decided difference between two forks when they were 36 vibrations apart. He could not describe the difference, but when the terms "coarse" and "fine" were suggested, he thought they expressed it very well. An hour's relaxation intervened between sets 4 and 5 and between sets 11 and 12.

The tests on myself (K. D.) were carried out by a student, who was not skilled in manipulating the forks. In many cases the vibration of one or both of the forks was so weak as to be barely perceptible. In set 1 I listened for the sound of the forks, but could hear it at no time. Even when my whole attention was given to the listening the sound was inaudible.

Where more than one set of tests were made on a subject, both hands were used. Some of the "A" sets were taken on the right hand of the subject, and some on the left. Some of the sets were taken in part on one hand and in part on the other. The maximal number of trials in succession on the same hand was fifty, and in many cases twenty-five was the limit. I kept a record of the hand in each case, but with every subject the results were practically the same on the two hands.

None of the subjects showed any pronounced tendency to errors of one sort rather than of the other. In other words, the subjects called the second fork "high" when it was really "low" about as often as they called it "low" when it was really "high."

The tendency to confuse pitch difference with intensity difference is quite marked. Most of the subjects finally find the lower pitch relatively rough or coarse, and the higher smoother or finer, but this specific difference is not constantly found by any subject. Often the two rates of vibration feel different in an indescribable way. It seems quite likely that in some cases the subjects invert the conventional terminology of "high" and "low," while still distinguishing between the sensations from the two forks (sets 4 and 5, K. D., for example).

My results seem to indicate that for normal persons the palmesthetic difference threshold for rate in the neighborhood of four hundred and forty vibrations per second is not above thirty-six vibrations and may be as low as sixteen vibrations. I feel certain that these values may be materially reduced by the use of forks which do not cease vibrating so quickly as do the small ones I used.

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THE PHYSIOLOGICAL ACTION OF EXTRACTS OF THE PINEAL BODY.

By H. E. JORDAN and J. A. E. EYSTER.

[From the Laboratories of Pharmacology and Histology of the University of Virginia and of Physiology of the University of Wisconsin.]

IN contrast to the large amount of work done on other organs of the body which are known or supposed to furnish an internal secretion, the pineal body has received little attention at the hands of the experimentalist. A number of papers have been published dealing with the normal and pathological structure of this organ, especially in regard to the occurrence and structure of pineal tumors, but we have been able to find only three references in the literature concerning its possible physiologic rôle. V. Cyon[1] found that the intravenous injection of extracts of the pineal body of the ox and sheep produced no effect on blood pressure; with weak doses the heart rate was increased, while with larger doses the beat was slower and stronger. V. Cyon concluded that the function of the pineal body was a mechanical one, serving to regulate the flow of cerebro-spinal fluid through the aqueduct. Howell,[2] in his work on the pituitary body, tested the effects of extracts of the cerebral cortex, medulla and pineal body, and found that the effects on blood pressure were not constant, some injections causing no change, others a considerable fall. He was interested mainly in the fact that the reactions obtained were essentially different from those obtained with extracts of the pituitary body, and did not further pursue the question of the action of these extracts. Dixon and Halliburton[3] tested the effects of extracts of the pineal body of sheep on arterial blood pressure, rate and depth of respiration, rate and force of heart beat, intestinal volume, kidney volume and rate of secretion of urine in the cat. One part of the dried gland was extracted with one hundred parts of cold or hot Ringer's solution, 0.1 per cent hydrochloric acid, or alcohol. One to 2 c.c. of these extracts produced no more effects than were obtained with the same amounts of Ringer's fluid. In most cases the injection of as much as 5 c.c. was entirely negative; the only effect observed was an occasional fall of blood pressure with the acid and alcoholic extracts. After the administration of atropine, the fall was replaced by a rise, which suggested choline. Chemical examination of the glands for this substance was, however, negative.

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[1] v. Cyon: Archiv für die gesammte Physiologie, 1903, p. 98, and Comptes rendus de l'Académie des Sciences, 1907, 144, p. 868.

[2] Howell: Journal of experimental medicine, 1898, iii, p. 245.

[3] Dixon and **Halliburton**: Quarterly journal of experimental physiology, 1909, ii, p. 283.

In view of the meagre experimental facts known, and the entire absence of any satisfactory knowledge regarding the function of the pineal body, it seemed to us that a careful testing of extracts of this organ in different animals might prove of some value. We have used in all of our experiments pineal bodies from sheep, because of the relatively large size in these animals and the readiness with which sheep brains could be obtained. The heads were kept on ice never longer than ten hours, until the brains were removed immediately before use. The pineal bodies were removed, and an extract made by rubbing up with clean sand and filtering or straining through gauze. In other cases pineal bodies which had been preserved for varying periods of time (up to three weeks) in alcohol or formaldehyde solution were carefully washed and extracted as above. The extract from one gland in 3 to 5 c.c. of salt solution was the usual amount injected at any one time. No difference in reaction was obtained between the extracts made from the fresh and the preserved glands.

The results obtained may be described as follows:

Effect on respiration. — Respiration was recorded by means of a transmitting tambour placed on the thorax in four experiments: two dogs, one cat, and one sheep. No striking or characteristic effects were noted in the anæsthetized animal. In the dog there was not infrequently a transitory decrease in size and increase in rate followed by a slowing and increase in depth (Fig. 1). In other cases (sheep) there was a gradual increase in depth of the respirations lasting for some time after the injection (Fig. 2).

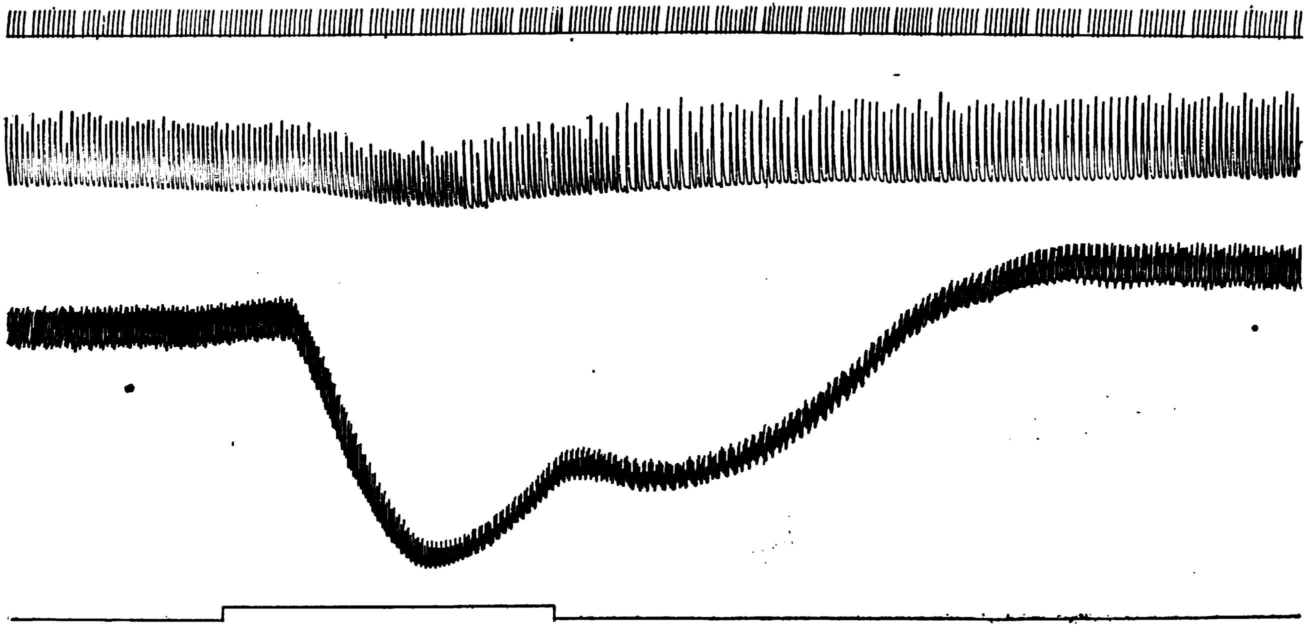

FIGURE 1.—Injection of neutral salt solution extract of one fresh pineal gland into the femoral vein of the dog. Top line in intervals of one second (every tenth second dropped). Second line from top, respiration; upstroke records inspiration. Third line, blood pressure from femoral artery. In the lowest line the rise marks the period of injection. The base line is omitted for lack of space. The mean arterial pressure before injection was 128 mm. Hg; during injection it fell to 50 mm. Hg.

dilatation. The fall in blood pressure was usually less than would be expected from the degree of intestinal dilatation, and the latter usually persisted for some time after the blood pressure had returned to normal. The results on blood pressure and intestinal volume were carefully controlled by injecting similar extracts made in the same way from various regions of the brains employed. While in some cases these extracts produced a slight fall of blood pressure, it was usually considerably less than that produced by the pineal extracts. Fig. 4 shows the effect of the injection of an extract made from a portion of the occipital cortex in the same animal immediately following the portion of the record from which Fig. 3 was reproduced. Extracts from various portions of all the brains used were tried with similar results.

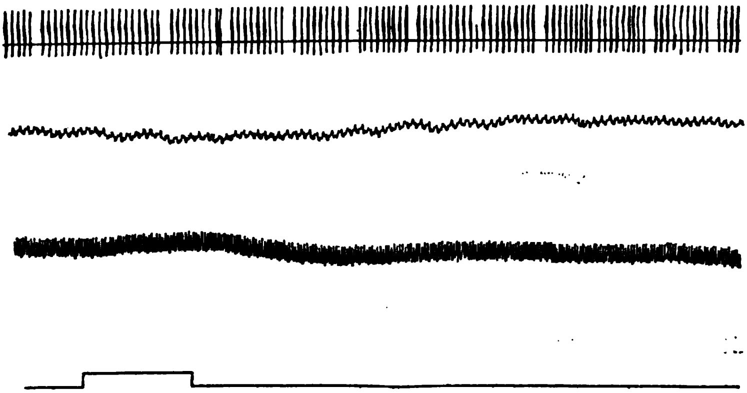

FIGURE 4. — Blood pressure and intestinal volume in a dog. Injection of neutral saline extract of a portion of the occipital lobe. Record to be interpreted as Fig. 3. The blood pressure before injection was 136 mm. Hg; after injection it varied between 138 and 133 mm. Hg.

Effect on excised heart. — In two experiments cats' hearts were perfused with Locke's blood solution by Langendorff's method. Different strengths of pineal extract were tested, varying from the extract of one fourth to one gland per litre of perfusion solution. The weaker solutions had no effect; the solution containing the extract of one gland to one litre of perfusion solution produced a slight increase in rate and in size of contraction, the latter amounting to 15 to 20 per cent. The flow through the coronaries was moderately increased when the stronger solutions were perfused, but this may be probably accounted for by the increased strength and rate of contraction. The lower line of Fig. 5 is a record of an excised cat's heart on pineal extract perfusion (extract of one gland to one litre of Locke's blood solution); the upper line records the beat on the solution without pineal extract.

Effect on secretion of urine. — A study of the effect of the intravenous injection of extracts of the pineal body on the amount of urine secreted was made in seven animals: six rabbits and one cat. Neutral salt solution extracts caused either no change or a considerable decrease in the amount of urine secreted. Extracts made with acidulated salt solution caused, on the other hand, in most cases a moderate but very transitory diuresis. The results were carefully controlled by the injection of the same amounts of acidulated salt solution. The blood pressure was recorded in one of these experiments (rabbit) and showed a slight rise following each injection of acidulated pineal extract. In one experiment the urine was increased about four times as the result of three injections of acidulated extracts. The diuresis is, as noted above, very transitory, lasting from three to ten minutes and usually followed by a slight reduction below normal. In the five experiments in which this point was tested, the urine following the injections of pineal extract reduced Fehling's solution in four; the normal urine before the injection of the extracts was negative in each case. The following is a protocol of an experiment:

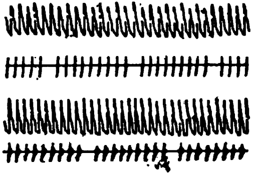

FIGURE 5. — Isolated cat's heart under artificial perfusion. Upstroke represents systole. Upper line shows beat on Locke's blood solution. Lower line on Locke's blood solution containing the extract of one pineal gland to one litre of perfusion solution. Time in intervals of one second.

Experiment of June 15, 1910. — Rabbit. Weight, 1910 gm. 20 c.c. urethane-chloral mixture by stomach tube at 9.00 A. M. No other anæsthetic used except ether for two short periods during the course of the experiment. Urine collected by bladder cannula. Blood pressure recorded from left carotid. Extracts injected into right external jugular vein. The pineal bodies used had been preserved for eight days in 95 per cent alcohol.

Experiment concluded. Normal urine does not reduce Fehling's solution. Urine collected after injection of pineal extract shows marked reduction of Fehling's solution.

Time.	Procedure.	Urine in drops in intervals of 5 minutes.	Blood pressure.
11.25	Normal	5.5	. . . .
11.30		5.5	
11.35		6.5	
11.40	Injection of neutral salt solution extract of one gland.	6.0	Considerable fall.
11.45		3.0	
11.52	Injection of neutral salt solution extract of two glands.	2.0	Very low. Artificial respiration for a short period.
11.57		1.0	
12.02		2.0	
12.08		6.0	
12.15	Injection of acidulated salt solution extract of one gland.	9.0	Slight rise.
12.20		10.0	
12.25		7.0	
12.30		7.0	
12.35	Injection of acidulated salt solution extract of one gland.	10.0	Slight rise.
12.40		8.0	
12.45		10.0	
12.50	Injection of acidulated salt solution extract of two glands.	23.0	Moderate rise.
12.55		12.0	
12.57	Injection of 4 c.c. acidulated salt solution.	5.0	No change.
1.02		5.0	
1.07		6.0	

SUMMARY.

Our experiments indicate that the pineal gland of the sheep contains some substance (or substances) which, on intravenous injection in certain animals, cause a fall of blood pressure associated with a vasodilatation in the intestines; produce a slight degree of improvement in the beat of the isolated cat's heart, and cause a transitory diuresis associated with glycosuria in about 80 per cent of the cases. We have found, in agreement with Dixon and Halliburton, that the effect on blood pressure in the cat is small and unimportant. It should be noted that our extracts were in all cases more concentrated than those employed by these investigators. On the whole, our work would seem to indicate that while certain definite effects on the circulation and secretion of urine are produced in certain animals as the result of intravenous injections of extracts of the pineal body, the action is relatively slight when compared with that produced by extracts from other glands known to furnish internal secretions.

Our experiments deal obviously only with a possible rôle of the pineal body in producing certain relatively rapid effects on the circulation, respiration, and secretion of urine. They leave entirely untouched the possibility of more gradual effects over longer periods of time, as well as the influence these bodies may exert on metabolism or other functions and their relation to other organs of internal secretion. From the experiments of Dixon and Halliburton, and those recorded in this paper, it seems clear that the first-mentioned influence, so far as it is exerted by any substance produced in the organ, must be relatively small and unimportant.

VISUAL SENSATIONS CAUSED BY CHANGES IN THE STRENGTH OF A MAGNETIC FIELD.[1]

By C. E. MAGNUSSON and H. C. STEVENS.

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THE discovery, recently made by S. P. Thompson,[2] that an alternating current magnetic field will arouse visual sensations deserves considerable attention. The observations have been repeated and extended by Knight Dunlap[3] at the Johns Hopkins University. Briefly, the maximal electro-magnetic conditions used may be summarized as in Table I.

In our own experiments, we were concerned with the following points:

1. To verify the results of Thompson and Dunlap.
2. To ascertain whether the magnetic field induced by the direct current gives a visual sensation.
3. To determine the threshold of the sensation in terms of ampere turns.
4. To determine the dependence of the sensation upon the frequency of the current.
5. To determine whether the magnetic field inhibits or reinforces a visual sensation.
6. To determine whether the magnetic field stimulates a nerve trunk.

Since the intensity of the sensations produced depends to a large extent on the intensity of the field, it was thought possible to produce similar results by using more turns of wire in the coil and hence avoid using large currents. This supposition was readily proved correct with a coil of 205 turns. Flickering light sensations were observed with a

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[1] Inasmuch as the authors are to be absent from the University of Washington for the ensuing year, it is deemed advisable to publish their experiments in the present incomplete form rather than to await the opportunity for further research.

[2] THOMPSON: Proceedings of the Royal Society, B 82 (557), p. 396.

[3] DUNLAP: Science, N. S., 1911, xxxiii, p. 68.

60 cycle current of 28 amperes, *i. e.*, 5740 ampere turns; a value approximately equal to the field intensity used by Thompson.

TABLE I.

Number of turns in coil.	Maximum current in amperes.	Maximum ampere turns.	Frequency.	Observer.
32	180	5760	50	S. P. Thompson.
27	200	5400	60	Knight Dunlap.
27	440	11,880	60	Knight Dunlap.
27	480	12,960	25	Knight Dunlap.

By increasing the number of turns the range of observations was extended although the currents were kept less than fifty amperes. With high insulation and still greater number of turns the field under investigation can readily be extended.

The conditions under which the data were taken may be described as follows:

A. **Coils.** — Two coils were used. The first had 205 turns of No. 16 B. & S. d. c. c. copper wire, and was elliptical in shape with the inside, minor and major, axes of 8.5″ and 12.0″, respectively. The second coil had 263 turns of No. 13 B. & S. d. c. c. copper wire and of dimensions (9.5″ and 13.5″) so as to slip on the outside of the first coil. In most of the experiments the second coil was kept on the outside of the first, giving 468 turns in series.

B. **Available electrical energy.** —

Alternating current.

	Frequency.	Volts.
Power House Bus-bars	60	240
Inverted Rotary Converter . . .	7–70	325
1 ϕ Generator	7–60	1100

Direct current.

	Volts.
Generator	650
Generator	125
Battery	260

C. **Observers.** — The writers were assisted by Mr. Enoch Karrer, Mr. Paul Kaylor, and Dr. L. O. Grondahl in reading the electrical instruments and in checking observations on the visual sensations.

D. **Position of coil and observer.** — The observer sat erect in a chair. The coil was suspended from a support directly above the observer, and unless otherwise noted, the plane of the coil was kept in a horizontal position. Hence the axis of the coil was in a vertical line passing as near as possible through the centre of the head of the observer.

The maximum effect for each field was obtained when the head of the observer was inside of the coil and placed so that the horizontal plane through the middle of the coil also passed through the upper edge of the eyes.

By raising the coil relative to the observer, the effect was diminished, and when the lower edge of the coil was in a plane with the top of the head or higher no effect was observed.

Lowering the coil from the central position also decreased the effect. When the coil touched the shoulders of the observer no effects were noticed. By shifting the head close to one side of the coil or by tilting the coil relatively to the head, variations in the intensity were noticed, but the observations were not sufficient to establish definite relations.

Most of the observations were made inside a small booth, open on top, in the electrical laboratory. In most cases the observer was carefully blindfolded. Excluding outside light seemed to be helpful while making observations on "threshold values" and for noticing variations in wave forms on the field of vision.

With the stronger fields the effect was readily noticed even in bright daylight and with the eyes open.

E. **Magnetic field.** — With the type of coil used the magneto-motive force, $F = 4\pi/10\ NI$, where NI are the ampere turns. Since the flux density depends on the magneto-motive force and the reluctance of the path for each line of force, the field inside the coil will not be uniform. It will be weakest along the axis perpendicular to the plane of the coil, as the reluctance for that part will be greatest. Since the reluctance for the lines near the centre of the coil increases with the diameter of the coil, more ampere turns are required to produce any given field intensity by both coils in series than by the smaller coil alone. Thus with direct currents (page 127) the threshold effect for the small coil was obtained by 7800 ampere turns, while with both coils 9300 ampere turns were required. No calculations were made on the field strength in terms of lines of force, as the results would at best be roughly approximate.

For a preliminary series it is deemed sufficient to give dimensions of the coil with the ampere turns used. Quantitative measurements in a uniform field could best be made with a coil in the form of a solenoid long enough to let one end rest on the shoulders of the observer and the other extending a foot or more above the top of the head. This would give a fairly uniform field whose value could readily be determined.

F. **Tables.** — The quantitative electro-magnetic values and the corresponding sensations noticed by the observer are for convenience given in tabular form.

TABLE II.

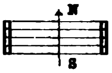
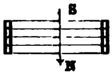
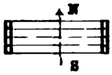
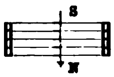
DIRECT CURRENTS.[1]

Volts.	Amperes.	Ampere turns.	Observer.	Observations.
48	17	3485	S.	Slight muscular contract'n in eyelids.
48	17	3485	M.	No effect.
48	17	3485	E. K.	No effect.
63	22.5	4613	M.	No effect.
63	22.5	4613	E. K.	No effect.
63	22.5	4613	S.	No effect.
120	38.5	7893	M.	Flash like a miniature sheet lightning on closing circuit.
120	38.5	7893	E. K.	Plain even flash on closing circuit. Muscular twitchings.
120	38.5	7893	S.	Weak flashes.
148	40	8200	S.	Weak flashes on closing circuit.
148	40	8200	E. K.	Flash on closing and on breaking circuit.
148	40	8200	M.	Flash on closing circuit very distinct; weaker on breaking the circuit.
147	42	8610	E. K.	Flashes very distinct. Stronger on closing than on breaking the circuit.

[1] In each case the circuit was closed and opened several times. Fatigue was noticed by all observers. The effect for a given number of ampere turns was greatest on closing the circuit the first time.

TABLE III.

Direct Currents. (For each Observation recorded in the Table the Circuit was closed and opened Several Times. The Current was passed through the Coil so as to make the North Pole above and the South Pole below the Plane of the Coil.)

Volts.	Amperes.	Ampere turns.	Observer.
North Pole above and South Pole below. 			
92	20	9360	M.[1]
Direction of Current was reversed through Coil. 			
93	22	10296	M.[2]
110	26	12168	M.[3]
110	26	12168	G.[4]
110	25.5	11934	E. K.[5]
The Direction of the Current was reversed through the Coil. 			
110	24.8	11606	M.[6]
110	24.7	11559	E. K.[6]
110	24.7	11559	G.[6]
208	39.5	18536	M.[7]
210	38	17784	G.[8]
215	41	19188	E. K.[9]
The Direction of the Current, and hence of the Field, was reversed. 			
220	42	19656	M.[10]
225	45	21060	E. K.[10]
225	45	21060	G.[10]
250	47.5	22230	M.[11]
255	52.0	24336	E. K.[11]
275	48.0	21464	M.[11]
...	...	....	...

[1] Faint flash only on closing the circuit. [2] Faint flash on closing the circuit.

[3] Flash stronger on closing than on breaking the circuit. The light appears as a narrow band or wave extending in a horizontal plane across the field of vision. The wave first appears at the upper part of the field of vision and moves rapidly downwards on closing the circuit. No effect noticed after the current had reached its full value. On breaking the current a fainter and less definite wave appears. The motion was not as definite as when the circuit was closed but appeared to move in an upward direction. [4] Faint flash on closing the circuit.

[5] Flash on closing the circuit appeared as a bright wave moving rapidly downwards across the field of vision.

[6] Both on making and breaking the circuit, wave on making moves downward.

[7] The wave of light on making the circuit moves rapidly downwards across the field of vision. The wave formed on breaking was weaker, and did not have as definite outline or motion. It appeared to start from below and move upwards.

[8] Same observations as above both as to relative intensity of the light wave and the directions of motion of the two waves.

[9] Similar effects as noted by M. and G. both as to relative intensity and motion of waves on closing and breaking the circuit.

[10] Wave bright with motion downwards on making the circuit. Wave weaker with motion upwards on breaking the circuit.

[11] Same as before except that the motion appeared to be faster than with weaker field.

The first column gives the E. M. F. in volts across the terminals of the coil. In the second column is given the amperes through the coil, while the third gives the corresponding ampere turns.

TABLE IV.

THRESHOLD VALUES. ALTERNATING CURRENTS.

Volts.	Amperes.	Ampere turns.	Frequency.	Observer.	Observations.
134	5.0	2340	60	M.	No effect.
175	6.0	2808	60	M.	No effect.
190	7.0	3276	60	M.	Slight effect.
198	8.0	3744	60	M.	Little quiver.
200	8.2	3838	60	M.	Two small areas in the temporal part of the field give a bright flickering light.
215	8.6	4025	60	M.	Slightly brighter.
194	7.8	3651	60	M.[1]	Uncertain.
138	5.8	2714	60	S.	No effect.
142	6.2	2902	60	S.	Very faintly.
139	5.9	2767	60	S.	Uncertain.
138	5.8	2714	60	S.	No effect.
141	6.1	3255	60	S.	Faint light.

[1] The readings by observer M. are taken from a set of forty-seven observations made to determine the threshold value. The increase and decrease was repeated four times. Fatigue was noticed as more excitation was required after observing for a short time.

In Table II the smaller coil with 205 turns was used, while in the other tables both coils with 468 turns were connected in series.

Under the heading "Observer" are given the initials of the person whose head was subjected to the magnetic field. Under "Observations" are given brief notes on the effects experienced by the "Observer." The "Frequency" is given in complete cycles per second.

SUMMARY FOR DIRECT CURRENTS.

(*a*) Visual sensations were produced by an increasing or decreasing field when the circuit was closed or opened. No effect was noticed when the current had reached a constant value.

(*b*) The effect appears as a narrow band or wave of light in a horizontal plane and rapidly moving downwards across the field of vision when the circuit is closed and upwards when the circuit is broken.

(*c*) The direction of the lines of force in the field (up or down) did not affect the direction of motion of the light wave.

(*d*) The wave observed on closing the circuit was brighter and more definite in outline than the corresponding wave when the circuit was broken. The rate of increase of field strength on closing the circuit was also greater than the corresponding decrease on opening the circuit. This tends to prove that the intensity of the visual sensation depends on the intensity and rate of change in the magnetic field.

(*e*) The threshold on closing the circuit was 7800 ampere turns, with the first coil (205 turns), and 9300 ampere turns when both coils (468 turns) were used. From the dimensions of the coils it will be seen that these two values give approximately the same field intensity.

In Table V observations were made at various field strengths under a considerable range of frequencies. It was planned to keep the current constant for each series and let the frequency be the single variable. The available controlling appliances were not well adapted for our purpose, so the adjustments were not as close as desired. The rapid heating of the coil and the fatigue of the observer made it necessary to make adjustments and take readings with all possible despatch.

The circuit was kept closed while changing the frequency of the generator. Hence the observer could note the effect at all frequencies within the limits given. While the frequencies were changing the current often varied in magnitude, and hence some of the variations noted were also affected by the change in field strength.

After the frequency had reached the value recorded in the table, the circuit was opened and closed several times The frequency was measured by an electrical Tachometer made by the Weston Electrical Instrument Co. With the larger currents the coil became very hot in a few minutes.

SUMMARY FOR ALTERNATING CURRENTS.

(*a*) With the coil used the threshold for current at 60 cycles was between 3000 and 4000 ampere turns.

These values, no doubt, vary considerably with different observers, and also with the physical condition of the observer during the test. The observer rapidly becomes fatigued, and, after the excitation has continued for a while, larger values are required to produce noticeable effects.

(*b*) Increasing the ampere turns also increases the intensity of the light sensations.

TABLE V.

ALTERNATING CURRENTS.

FIRST SET. TIME FOR FIRST SET, NINE MINUTES.					SECOND SET. TIME FOR SECOND SET, ELEVEN MINUTES.				
Volts.	Amperes.	Ampere turns.	Frequency.	Observer.	Volts.	Amperes.	Ampere turns.	Frequency.	Observer.
122	12.2	5710	19.8	M.[1]	108	11.4	5335	18.7	M.[6]
186	12.8	5990	32.0	M.[2]	230	12.6	5897	44.0	M.[7]
208	10.1	4763	46.6	M.[3]	280	10.5	4914	60.2	M.[8]
192	12.5	5850	32.6	M.[4]	210	10.4	4867	44.7	M.[9]
120	11.7	5476	20.0	M.[5]	100	10.0	4680	20.0	M.[9]

[1] Flicker at first quite bright, soon becomes weaker.

[2] Bright flicker with distinct bands of light or standing waves, in temporal part of the fields in both eyes. The effect was brightest while the frequency was changing from 20 to 30 cycles and became less after the constant frequency of 32 cycles had been reached.

[3] The light decreased both in intensity and distinctness of the waves as the frequency increased. (It should be noted that the current also decreased.)

[4] While reducing the frequency the light increased in brightness and the bands or waves again became clear and definite in outline.

[5] On reducing the frequency the light became less intense. The effect at this stage was considerably less than at the beginning of the series with approximately the same frequency and current. The difference is supposed due to fatigue of the observer.

[6] The waves and the flickering sensations were much the same as in the first series.

[7] On changing frequency very bright waves appeared in the temporal part of the field of vision, for both eyes.

[8] The effect was weaker at this frequency.

[9] Faint light.

TABLE V — *Continued.*

Third Set. Time for Third Set, Sixteen Minutes.					Fourth Set. Time for Fourth Set, Nine Minutes.				
Volts.	Amperes.	Ampere turns.	Frequency.	Observer.	Volts.	Amperes.	Ampere turns.	Frequency.	Observer.
80	11.9	5579	10.3	M.[1]	80	10.8	5054	11.3	E. K.[6]
144	12.8	5990	21.3	M.[1]	104	11.9	5579	15.8	E. K.[6]
162	12.4	5803	26.7	M.[2]	150	13.0	6084	23.3	E. K.[6]
184	12.3	5756	32.3	M.[2]	242	13.8	6858	38.0	E. K.[6]
272	12.4	5803	46.7	M.[3]	274	14.0	6552	43.3	E. K.[7]
320	10.8	5054	63.3	M.[4]	312	14.0	6552	48.6	E. K.[7]
262	13.0	6084	43.3	M.[5]	348	14.0	6552	54.0	E. K.[7]
130	12.0	5616	23.3	M.[5]	354	12.8	5990	60.8	E. K.[7]
80	9.6	4493	10.0	M.[5]	356	12.0	5616	65.8	E. K.[8]
..	..	..	..	..	120	12.0	5616	17.6	E. K.[9]
..	..	..	..	..	84	9.6	4493	12.0	E. K.[9]
..	..	..	..	..	76	9.1	4259	7.5	E. K.[9]

[1] At start the light was a bright shimmer over the whole field, rising and falling in intensity with the frequency. The pulsations were of about the same period as the frequency and could readily be counted. As the speed of the generator was increased the pulsations were faster, and at about 20 cycles the observer was unable to note the individual rise and fall of each cycle. Above this frequency there appeared a set of meshes of light or a combination of standing waves, strongest in the temporal part of the field.

[2] The meshes decreased in size and also in sharpness of outline as the frequency was increased.

[3] Increasing the frequency over 40 cycles, the mesh or standing wave formation disappeared, and the flickering became very rapid with a more uniform distribution of the light. The effect also was weaker.

[4] The effect was weaker, but the same appearance as at 46.7 cycles.

[5] On decreasing the frequency the series of changes noted above were repeated. The standing waves were brightest between 30 and 20 cycles. The effect was much weaker than at the beginning of this set. The difference was probably due to fatigue of the observer.

[6] Flashes distinctly of the same period as the frequency of the current and field. As the frequency increased the illuminated field divided into two parts. The light seemed brighter when turning the eyes to extreme right or left.

[7] Meshes or standing waves were distinct at 20 cycles and above. At the higher frequencies the effect was a continuous illumination with very rapid flickering.

[8] At the highest frequency the effect was a steady constant light.

[9] On lowering the frequency the effect was much weaker. Hardly any effect could be noticed for the last two sittings.

TABLE V — *Continued.*

FIFTH SET.
TIME FOR FIFTH SET, TWELVE MINUTES.

Volts.	Amperes.	Ampere turns.	Frequency.	Observer.
106	13.8	6858	14.0	E. K.[1]
146	14.4	6739	20.0	E. K.[2]
164	14.9	6973	22.0	E. K.[3]
228	15.9	7441	30.6	E. K.[3]
254	13.0	6084	43.3	E. K.[3]
272	13.1	6131	46.6	E. K.[4]
280	12.1	5663	52.0	E. K.[5]
292	12.3	5756	52.6	E. K.[5]
324	12.3	5756	60.0	E. K.[6]
350	12.4	5803	63.3	E. K.[6]
362	12.7	5993	65.3	E. K.[6]

SIXTH SET.

Volts.	Amperes.	Ampere turns.	Frequency.	Observer.
360	29.0	13572	27.4	M.[7]
360	27.5	12870	27.4	P. K.[7]

SIX SET — *Continued.*

Volts.	Amperes.	Ampere turns.	Frequency.	Observer.
300	30.5	14274	19.5	M.[8]
290	30.0	14040	19.5	P. K.[9]
280	31.0	14508	15.5	M.[10]
260	30.2	14134	12.5	P. K.[11]
220	30.0	14040	8.0	M.[12]
210	30.0	14040	9.7	P. K.[13]
210	30.0	14040	11.0	P. K.[13]
420	29.5	13806	31.0	M.[14]
410	29.0	13572	31.0	E. K.[15]
400	29.0	13572	31.0	P. K.[16]
455	28.0	13104	39.5	M.[17]
540	30.5	14274	39.0	M.[17]
535	30.0	14040	39.0	E. K.[17]
615	30.0	14040	46.0	M.[17]
620	30.0	14040	46.0	E.K.[17]
615	30.0	14040	46.0	P. K.[17]
615	30.0	14040	46.0	G.[17]

[1] Distinct flicker of pulsation.

[2] Field appears in three parts, a fairly bright centre and brighter spots in the temporal regions.

[3] Distinct mesh or standing wave formation with flickerings. The meshes decreased in size with increase in frequency. [4] Effect fainter and more uniform.

[5] Distinct twitchings of eyelid muscles when eyes were turned to one side.

[6] The effect was much fainter toward the close of the set, and for the last frequency it almost disappeared.

[7] Field very bright and filled with a network of standing waves. The whole field quivered or flickered continuously.

[8] The frequency started at 31.0 and was reduced to 19.5. The waves were not so distinct as above. The whole field also appeared to have considerable diffused light.

[9] The same reduction from 31.0 to 19.5 was repeated. The same effect was observed.

[10] The frequency was changed from 25.5 to 15.5 cycles. The field was bright on starting with distinct meshes or standing waves. As the frequency dropped the distinctness of the waves faded, and at the lower frequency the light was quite uniform over the field, and flickered.

[11] The frequency was changed from 24.7 to 12.5. At first the waves were standing clear and bright, but as the frequency was reduced the light was dimmed. At the lower frequency the effect changed from standing waves to a flickering effect.

[12] The whole field pulsates or flickers somewhat like sheet lightning.

[13] Whole field flickers or pulsates.

[14] Very bright standing waves. Brightest in temporal part of the field. After a few seconds the light in the central part of the field faded and became very faint while the temporal portions remained bright.

[15] Bright steady waves all over field.

[16] Bright steady waves all over field. After a few seconds the central portion fades and becomes faint.

[17] Very bright standing waves. The field was divided into three bright areas, one in the centre and one on each side. In between these areas the light was diffused and comparatively weak. Central part slowly becomes weaker while the temporal areas remain bright.

(*c*) The frequency of alternations has a marked effect on the visual sensations. With the same strength of field the effect appears greatest between 20 and 30 cycles per second.

At frequencies below 15 per second the light pulsates in a succession of flashes over the whole field. From 20 to 35 cycles the light appears as a network of standing waves on which is superimposed a quivering flickering effect. The size of the meshes is smaller for the higher frequency. Above 40 cycles the light becomes more uniform and the flicker more rapid.

(*d*) The effect is the greatest in the temporal parts of the field. In this connection it is interesting to recall Schoen's experiments,[4] upon the sensitivity to light intensities of the nasal and temporal halves of the retina, in which he found that the nasal retina was more sensitive than the temporal retina.

(*e*) Effects on *m. orbicularis oculi:* Slight twitchings in this muscle were noticed by several observers. It was thought that this contraction might exert pressure on the eyeball and through it on the retina and so cause the visual sensation. This view was negated by two facts: (1) The twitching soon disappears even in those observers in whom it was most marked, while the light sensation continues. (2) The twitching does not occur at all in some observers, while the visual sensation is very apparent.

(*f*) No sensation other than visual was noticed by any of the observers. No after effects appeared. For still stronger fields, care should be exercised, as the sensations are of considerable intensity.

OBSERVATIONS WITH AFTER IMAGES.

It was suggested by Dunlap that the visual sensations produced by the magnetic field were possibly due to the inhibition or reinforcement of some visual excitation going on in the nerve at the time of stimulation by the magnetic field. To obtain evidence bearing on this matter, some observations were made with after images. It was assumed that the visual after image is caused by a persistent excitation of the retinal or cortical neurones after the cessation of the stimulus. The visual after image would, therefore, fulfil the conditions of Dunlap's suggestion, unless he meant a subliminal excitation. An after image of an incandescent lamp was therefore obtained, the head of the observer was placed in the coil and the current turned on. The results, which are given in Table VI, show that the magnetic field had no apparent influence upon the after image.

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[4] SCHÖN: Archiv für Ophthalmologie, 1876, xxii, abth. 4, p. 31; also 1878, xxiv, abth. 4, p. 47.

TABLE VI.

Volts.	Amperes.	Ampere turns.	Frequency.	Observer.	Volts.	Amperes.	Ampere turns.	Frequency.	Observer.
124	13.2	6178	19.3	E. K.[1]	122	12.7	5944	19.3	P. K.[3]
124	12.9	6037	19.3	E. K.[2]	122	12.6	5897	19.3	P. K.[3]
122	13.0	6084	19.3	M.[3]	...	...	....	...	...

[1] No effect upon after image.

[2] The light produced by the magnetic field appeared much the same as when no after image was present.

[3] Strong after image produced by an incandescent lamp. The light sensation from the magnetic field was not affected by the after image. The flickering light passed over the after image and still left it distinct.

EFFECTS ON THE SCIATIC NERVE OF A CAT.

The sciatic nerve of a young cat was exposed after anæsthesia and tracheotomy, and placed between the poles of an electro magnet which gave a strong magnetic field. The iron core was in the shape of a horseshoe with the ends 1 cm. apart. The pole area was a rectangle 1 cm. by 5 cm. The flux density, while not measured directly, was near the saturation point of the iron. A 60-cycle current was applied which gave a rapidly alternating field of considerable intensity. This was the only frequency used for this experiment. The nerve was placed at the inner edge of the poles, so that most of the lines of force in the rapidly changing field would cut the nerve. The results were negative. Some twitchings of the muscles in the leg were noticed, but these could not be reproduced at will by applying the magnetic field. Unfortunately, time did not permit the repetition and extension of this experiment upon other animals and upon other nerves.

It was hoped to determine by this experiment whether the locus of the excitation in the production of visual sensations is in the sensory elements of the retina itself or in the fibres of the optic nerve. The observation must be extended before definite conclusions can be reached.

THE PERIPHERAL RESISTANCE AS A COMPENSATORY FACTOR IN THE POST-HEMORRHAGIC RECOVERY OF BLOOD PRESSURE

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SINCE Hales's [1] pioneer experiments, it has been repeatedly shown that the withdrawal of blood causes a lowering of blood pressure; from which, as Tappeiner [2] pointed out in 1872, a more or less complete recovery takes place, provided the loss of blood has not been too great. The conclusion that this recovery is mainly due to a compensatory constriction of blood vessels has been almost unanimously accepted by physiologists and clinicians alike. The idea seems originally to have been suggested by experimental results obtained in the studies of the opposite condition, namely, plethora. Throughout the early literature, as in the account of Tigerstedt,[3] so often quoted, attention is called to the transfusion experiments of Worm-Müller, Magendie, Poiseuille, Pawlow, and others. These investigators showed that an increase in the fluid content of the blood resulting from a transfusion caused no permanent increase in blood pressure, a phenomenon which could not be adequately explained by an increased secretion and lymph formation alone.

Since the work of the Leipzig Laboratory of Professor Ludwig had demonstrated the existence of a nervous mechanism governing the size of blood vessels which tended to keep the pressure at a constant level, Worm-Müller,[4] and many others after him, held it at once necessary and logical to assume that a dilation of peripheral blood vessels and consequent lowering of resistance was a factor of the greatest importance in equalizing blood pressure during plethora. It was but a step to formulate a corollary and apply it to the return of blood pressure after hemorrhage. "After withdrawal of blood," says Tigerstedt,[3] following a discussion of plethora, "the processes operate in a reverse manner. The heart empties itself as completely as possible, ejecting the largest possible quantity of blood into the blood vessels, at the same time that the latter constrict and increase the resistance to the flow of blood."

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[1] HALES: Haemostatiks, 1733, ii, p. 2.

[2] TAPPEINER: Berichte der sächsichen Gesellschaft der Wissenschaften (Math.-phys. Classe), 1850, p. 199.

[3] TIGERSTEDT: Lehrbuch der Physiologie des Kreislaufes, Leipzig, 1893, p. 349.

[4] WORM-MÜLLER: Berichte der sächsichen Gesellschaft der Wissenschaften (Math.-physik. Classe), 1850, p. 192.

Imbued with these logical explanations, various investigators have sought to advance direct experimental proof that such a constriction occurs. Klemensiewicz[5] showed that the loss of blood diminished the calibre of blood vessels in a frog's foot, as observed under a microscope. Even granting, however, that the reactions of cold and warm blooded animals may be compared in this manner, the method fails to demonstrate that the change was not passive and due to the reduced volume of blood.

Crile,[6] in a long series of experiments, presents what appears to him evidence that the activity of the vaso-motor centre alone controls the post-hemorraghic rise of blood pressure; that, in fact, when no such rise occurs, the centre is exhausted. The proofs submitted in evidence are as follows:

1. A dose of strychnine causes a rise in blood pressure in an animal bled to such a degree that no post-hemorrhagic rise occurs without such an injection. Even if the statement of Crile that the drug is without action on the heart, remains unchallenged,[7] it is still difficult to conceive by what reasoning this may in any way be considered proof that the post-hemorrhagic rise is due to a similar action.

2. This post-hemorrhagic rise is less marked if the animal has previously suffered from shock, malnutrition, or disease. The evidence of this observation rests upon what Crile considers a demonstrated fact, *viz.*, that the vaso-motor centre may be "depressed" or "exhausted" by these conditions. The observations of Porter,[8] as well as those of Lyon

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[5] Klemensiewicz: Sitzungsberichte der königlichen Akademie der Wissenschaften, Wien, 1887, xcvi, Abth. iv. s. 57.

[6] Crile: Hemorrhage and transfusion, pp. 64 *et seq.*

[7] Hirschfelder: Diseases of the heart and aorta, 1910, p. 181.

[8] Porter: This journal, 1906, xxi, p. 460.

and Seelig,[9] however, indicate that some exception may be taken to such a statement.

3. When a recovery of blood pressure occurs after hemorrhage in dogs, burning of a paw accelerates this recovery; but when no post-hemorrhagic rise naturally takes place, the burning reflex is also absent. On the contrary, Porter [8] has been able to obtain a reflex rise of blood pressure in cats and rabbits, even when the blood pressure has fallen nearly to zero.

Sollmann and Pilcher [10] have briefly reported what may prove, when more details are available, definite evidence of an activity of the vaso-motor centre during hemorrhage. These investigators perfused a spleen, left in contact with the central nervous system, and obtained a decreased flow of fluid during hemorrhage. This, however, does not solve the entire question, as to whether the total peripheral resistance is actually increased during hemorrhage. Peripheral resistance as a factor influencing blood pressure contains more elements than constriction alone. The viscosity of the blood and the venous pressure are factors that may not be neglected in the study of this subject. It is entirely conceivable that as far as the effect on arterial blood pressure is concerned, a slight constriction, due to vaso-motor activity, might be entirely neutralized by a decrease in viscosity or a lowered venous pressure, both of which are known to exist in hemorrhage. There is, therefore, no undisputed evidence that an increase in peripheral resistance occurs after hemorrhage, much less that it is the chief, or even one of the causes of the post-hemorrhagic return of blood pressure. We refer here to the physiological rise of pressure following hemorrhage; which must be carefully distinguished from the slight mechanical rise which Dawson [11] has shown to follow immediately after the application of a clamp to the vessel from which the blood is drawn. On the other hand, experimental facts are available to show that the heart may contribute materially to the increase in pressure. This is brought about by an increase in the minute output following an accelerated rate, and an increased diastolic filling which follows the cessation of hemorrhage.

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[9] Lyon and Seelig: Journal of the American Medical Association, 1909, lii, p. 45.

[10] Sollmann and Pilcher: This journal, 1910, xxvi, p. 233.

[11] Dawson: Journal of experimental medicine, 1905, vii, p. 1.

According to the experiments of Wiggers,[12] the post-hemorrhagic rise of arterial pressure is always associated with such an augmented filling, and when no rise of pressure occurs, the increase in post-hemorrhagic filling is less pronounced. There seems, therefore, to be no question that the heart contributes something to the return of blood pressure.

In view of these facts, an investigation of the changes of peripheral resistance occurring after hemorrhage, and the part it plays as a compensatory mechanism in the process, was undertaken. This paper is to be considered merely a preliminary report of the total changes in the resistance; which comprises the resultant of the vaso-motor changes (central and direct), changes in the viscosity of the blood, and resistance offered by the venous pressure. A more detailed study as to how these factors interact has been planned for the near future.

METHOD.

The principle of the method utilized to study variations in the total resistance is very simple in theory, and works precisely in physical experiments used to test it out. If a large artery is compressed by a clamp, the pressure (recorded by a mercury manometer from one of its branches peripheral to the clamp) will fall from one level to another, at a rate varying directly with the total resistance offered to the outflow of the blood through the other branches peripheral to the clamp. If the total peripheral resistance remain the same, the time it takes the pressure to fall from 40 to 30 mm., from 30 to 20 mm., and from 20 to 10 mm., respectively, should remain approximately the same during different observations. If the *total peripheral resistance decrease*, it should fall more rapidly; if it *increase*, the fall should be less rapid. This method was suggested by Marey,[13] in 1882.

Practical application of the principle to animals, in which the resistance was changed by adrenalin, nitro-glycerine, and stimulation of the central end of the vagus, showed the principle held true, provided certain conditions were fulfilled, *viz.*:

1. The resistance between the cannula and the mercury manometer

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[12] WIGGERS: Archives of internal medicine, 1910, vi, p. 281. Also unpublished experiments.

[13] MAREY: La circulation du sang, 1882, chap. xii, p. 120.

must not change during the experiment. To this end the cannula and connecting bulb must be firmly fixed, and clot formation absolutely avoided. The latter was accomplished by using cannulæ dipped in a saturated solution of wax in ether, the cannulæ having been previously cleaned by passing successively through sulphuric acid, acetic acid, and alcohol.

2. Collateral circulation between points above and below the point of application of the clamp must be prevented.

3. The compression must not materially alter the pressure in the vessels supplying the brain; otherwise an alteration of cerebral blood supply might affect the vaso-motor centre independently of the hemorrhage.

After a considerable amount of preliminary experimentation, the following technic was found the most available one that would comply with the above requirements. The dogs used in all these experiments were anesthetized, either by morphine and chloretone, or by chloretone alone. The chloretone was dissolved in warm olive oil and injected intraperitoneally through a veterinary syringe. A carotid cannula was inserted for withdrawal of blood, and a second, waxed cannula was inserted into a femoral artery (after ligating the *profunda femoris artery*), and connected with a mercury manometer.

In spite of the momentum introduced by so cumbersome an instrument as the mercury manometer, it was nevertheless preferred, for the following reasons: 1. The needle writes in a vertical line, not an arc; 2. The tension of the writing point on the drum is constant; 3. The blood pressure may be read directly from the drum without preliminary calibration; 4. The results are uniform, as there are no variations due to changes in the elasticity of a membrane; and 5. When properly dampened, there is no throw, nor friction variation.

Through an abdominal incision the *sacralis media* and hypogastric arteries were next ligated *en masse*, and a long-handled clamp was applied to the aorta, just above its bifurcation into the femoral arteries. The collateral saphenous branch proved too insignificant to require attention. The abdomen was then closed by a few stitches, and the clamp manipulated from the outside.

When these procedures were completed, the femoral pressure, together with a time signal marking one-tenth seconds, and a zero line, were recorded on a drum revolving at approximately 3–4 cm. per second.

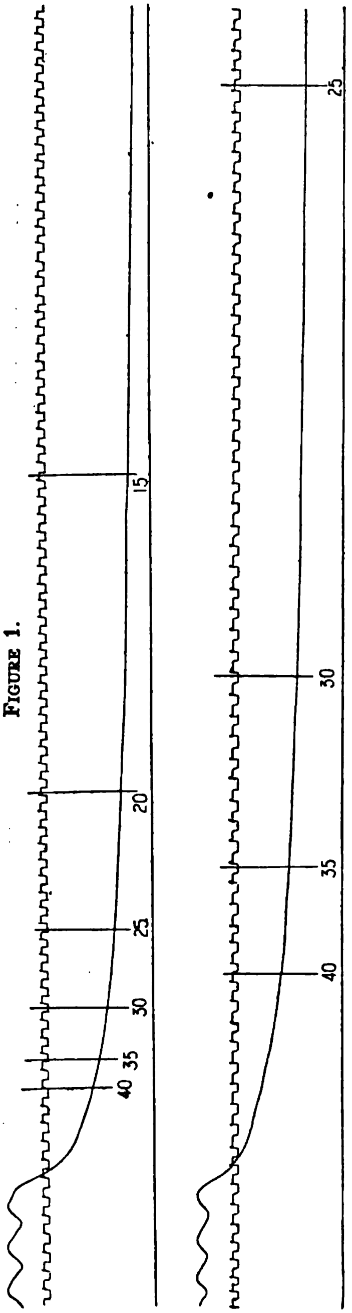

FIGURE 1.

When the clamp was suddenly closed, so that the aorta was completely shut off, the manometer record fell, describing the curve of parabolic form, finally approaching the horizontal (Fig. 1). After shellacing the record, the time required for the pressure to fall a stated distance was determined, and expressed in fractions of a second. This procedure was repeated in the following order:

1. The aorta was compressed twice, to obtain normal curves.

2. Blood was drawn from the carotid artery in varying amounts and at varying rates. The change in the blood pressure was shown on a slow drum.

3. During the hemorrhage and at varying intervals afterward, the aorta was again compressed.

4. A constricting or dilating drug was tested as a check wherever possible.

5. At the close of the experiment the femoral and cannula were examined, and if either contained an adherent clot, the results were disregarded.

RESULTS.

Out of twenty-seven attempts, thirteen experiments yielded good results, the others being marred by mishaps of one kind or another. As the results are not uniform, it will be necessary to analyze them in protocol form.

PROTOCOLS.

Experiments illustrating cases in which the peripheral resistance was increased, either during or shortly after hemorrhage:

EXPERIMENT No. 4 A. FEB. 17, 1911. DOG. WEIGHT, 4 KILO.

Time. P. M.	Initial pressure. mm.	Rate of pressure fall.		
		40–30 mm.	30–20 mm.	20–10 mm.
4.52	62.0	0.511	1.427	3.866
4.56	Hemorrhage, 200 c.c.			
5.01	46.5	1.089	3.162	....
5.06	26.0	2.999	....	....
5.34	35.5	1.578	3.177	....

EXPERIMENT No. 6 B. FEB. 28, 1911. DOG. WEIGHT, 13 KILO.

1.50	76.5	2.8	7.8	....
1.52	Hemorrhage, 100 c.c.[1]			
1.53	62.5	4.1	11.5	....
1.56	72.0	2.4	8.9	....
2.02	72.5	2.9	11.8	....
2.08	69.0	3.1	17.3	....
2.11	55.0	2.5	15.1	....
2.15	Hemorrhage, 100 c.c.			
2.24	53.0	2.2	11.5	....
2.32	46.5	2.2	8.7	....
3.13	54.0	2.0	7.2	....
3.19	50.0	1.5	3.4	....

EXPERIMENT 18. MAY 5, 1911. DOG. WEIGHT, 14 KILO.

12.45	64.5	1.23	2.87	....
12.56	Hemorrhage, 100 c.c.			
12.58	63.0	1.74	3.59	....
1.10	53.0	1.34	3.69	....
1.12	Hemorrhage, 100 c.c.			
1.13	47.0	1.82	4.65	....
1.30	45.0	0.93 (?)	4.49	....
1.32	Hemorrhage, 100 c.c.			
1.34	33.0	....	6.1	....
1.43	31.5	....	5.02	....
1.53	Saline infusion, 100 c.c.			
1.57	30.0	....	3.66	....
2.00	32.0	....	2.90	....

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[1] Slowly drawn.

EXPERIMENT 20. MAY 11, 1911. DOG. WEIGHT, 11 KILO.

Time P. M.	Initial pressure. mm.	Rate of pressure fall. 40–30 mm.	30–20 mm.	20–10 mm
1.15	82.0	1.62	....	....
1.17	86.0	1.60	....	....
1.22	Hemorrhage, 100 c.c.			
1.25	59.5	7.72	....	....
1.39	Hemorrhage, 100 c.c.			
1.41	41.0	5.45	....	....

EXPERIMENT 22B. MAY 19, 1911. DOG. WEIGHT, 7 KILO.

Time P. M.	Initial pressure. mm.	40–30 mm.	30–20 mm.	20–10 mm
12.21	74.0	1.99	5.3	14.57
12.25	Hemorrhage, 75 c.c.			
12.26	70.0	2.01	6.93	....
12.27	55.0	2.39	6.29	20.6
12.43	Hemorrhage 75 cc.			
12.44	48.0	0.88	1.89	6.02

EXPERIMENT 23. JULY 17, 1911. DOG, WEIGHT 11 KILO.

Initial pressure.	Rate of pressure fall. 40–35 mm.	35–30 mm.	30–25 mm.	25–20 mm.	20–15 mm.
55	0.06	0.10	....	....	....
70	0.055	0.08	0.16	4.2	7.6[1]
45	1.10	1.7	2.5	7.8	17.1
62	1.40	2.3	5.1	9.6	29.0
60	2.40	2.4	5.6	10.8	38.0

EXPERIMENT 27. JULY 23, 1911. DOG. WEIGHT, 13 KILO.
(See Fig. 1.)

Initial pressure.	40–35 mm.	35–30 mm.	30–25 mm.	25–20 mm.	20–15 mm.
75	2.8	4.0	6.0	9.2	10.9
75	1.8	3.0	4.5	10.0	18.0 (?)[2]
110	10.5	9.3	8.5[3]	....	....
98	1.7	3.1	5.0	8.5	19.0
85	2.3	3.2	5.0	10.5	19.0[4]
94	5.5	9.0	10.2	....	....

[1] Hemorrhage, 150 c.c.

[2] Stimulation of central end of vagus.

[3] Stimulation of vagus discontinued.

[4] Hemorrhage, 175 c.c.

SUMMARY.

Of the twelve cases of hemorrhage tabulated above, nine show an increase in total resistance following the loss of blood, the other three showing a decrease (the former shown by a slower rate of pressure fall, the latter by a more rapid rate). Of the nine cases, four were followed by a compensatory rise of arterial pressure, but in four no such reaction followed. Of the three cases showing a decreased peripheral resistance after loss of blood, in only one was there a compensatory rise.

From these experiments it may be concluded that while the increase in peripheral resistance plays an important part, it is by no means the only factor in the return of blood pressure after hemorrhage. Since the total peripheral resistance increases in spite of the decreased viscosity and lowered venous pressure, it may be inferred that a constriction of arterioles is the cause of this reaction, though more direct evidence of this is demanded.

An increased peripheral resistance, as shown in the preceding cases, is not always found, as is shown in the following experiments on animals with a low initial pressure:

EXPERIMENT 25. JULY 19, 1911. DOG. WEIGHT, 8 KILO.

Initial pressure.	Rate of pressure fall.				
	40–35 mm.	35–30 mm.	30–25 mm.	25–20 mm.	20–15 mm.
48	0.32	0.63	0.88	2.14	4.0 (?)[1]
48	0.36	0.63	1.12	2.22	....
34	....	0.47	0.53	1.82	6.3
37	....	0.275	0.60	1.48	5.1[2]
28	....	....	0.275	0.275	0.9
EXPERIMENT 24. JULY 19, 1911. DOG. WEIGHT, 9½ KILO.					
56	0.55	0.73	1.35	2.90	4.8
57	0.51	0.81	1.47	3.20[3]	....
36	....	....	....	....	....
42	0.20	0.34	0.40	0.80	....
EXPERIMENT 26. JULY 22, 1911. DOG. WEIGHT ?					
42	0.19	0.37	1.15	3.05	....
45	0.35	0.52	1.12	2.50[4]	....
25	....	....	....	1.25	2.75
30	....	....	0.35	0.87[5]	1.85
23	....	....	....	1.48	1.23

[1] Hemorrhage slow.

[2] Nitroglycerine 1/100.

[3] Hemorrhage, 160 c.c.

[4] Slow hemorrhage.

[5] Nitroglycerine.

In all the above experiments the first withdrawal of blood was followed by a pronounced decrease in peripheral resistance, as indicated by the more rapid rate of pressure fall. In none of these experiments did the compensatory rise of pressure equal more than 6 mm. These experiments indicate that when the total peripheral resistance decreases after hemorrhage, the blood pressure does not recover. They supply no evidence, however, that the low resistance is due to the relaxation of blood vessels, nor do they necessarily indicate a failure of the vasomotor centre. This is only one possibility, the others being that the venous pressure and the viscosity of the blood have been so much reduced that the constriction cannot overcome those factors.

CONCLUSIONS.

1. The total peripheral resistance offered to the flow of blood involves three factors, — the size of the peripheral arterioles, the viscosity of the blood, and the venous pressure.

2. The total peripheral resistance increases after the withdrawal of blood in many animals that show a post-hemorrhagic recovery in blood pressure. This indicates that an increased peripheral resistance is one, though not the only, mechanism concerned in the recovery.

3. Since the viscosity of the blood and the venous pressure both decrease and tend to reduce the resistance, the increase in the total resistance observed must result from a peripheral vaso-constriction.

I wish to express my sincere thanks to Dr. C. J. Wiggers for his suggestions and criticisms during this work.

THE TEMPERATURE COEFFICIENT OF THE RATE OF REPRODUCTION OF PARAMÆCIUM AURELIA.

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VITAL phenomena are undoubtedly largely the result of chemical reactions taking place in the living substance, and consequently one would expect that the well-known law of van't Hoff and Arrhenius, in regard to the velocity of chemical reactions in relation to temperature, should apply, *i. e.*, for each increase of 10° C. in temperature the rapidity of a reaction is increased from two to three times. Considerable data have been accumulated in regard to the effect of different temperatures on living processes in general, and on the rate of reproduction of infusoria, but comparatively little work has been undertaken with special reference to the temperature coefficient. Snyder made a summary of the chief results of the temperature determinations of physiological activities published prior to 1908, and he computed the coefficients in those cases in which it had not already been done. It is evident that "the coefficients for the most part, and for those physiological activities where we know (because of other reasons) metabolism takes place, are of a magnitude of the coefficients for chemical reactions."[1] More recent investigations, for example, by Riddle[2] on the process of digestion in cold-blooded vertebrates, and Rogers[3] on the rate of heart beat in various living vertebrates and invertebrates, point in the same direction.

There are few data extant in regard to the coefficient of cell division. Peter[4] found that cleaving echinoderm eggs gave a coefficient of 2.1, and computations by Cohen from the data of O. Hertwig[5] on developing frog's eggs showed a coefficient of 2.7. A thorough study has not been made of the temperature coefficient of cell division in the protozoa; in fact, none of the physiological processes of unicellular animals have been directly attacked from this standpoint, except the rate of pulsation of the contractile vacuole of several species of infusoria, chiefly by Kanitz.[6] The published data on the effects of temperature on the reproduction of infusoria are not sufficiently comprehensive to allow the computation of coefficients of value. Borowsky,[7] in a study of Actinosphærium, gave a brief table of the effect of different temperatures on the multiplication of this form, and from these data we have computed the coefficient and find it to be 2.63.

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[1] Snyder: This journal, 1908, xxii, p. 309.

[2] Riddle: This journal, 1909, xxiv, p. 447.

[3] Rogers: This journal, 1911, xxviii, p. 81. Our thanks are due to my friend Professor Rogers for a suggestive discussion of this problem. — L. L. W.

[4] Peter: Archiv für Entwickelungsmechanik, 1905, xx, p. 130.

The object of the present investigation is to make a detailed study of the effect of several constant temperatures on the rapidity of cell division in Paramæcium aurelia, with especial reference to the temperature coefficient. The ease with which paramæcia lend themselves to experimental treatment makes them one of the most favorable forms for general physiological study, and the fact that these animals consist of but a single cell eliminates many complications which arise in work with higher forms. For example, considerable differences exist in the effect of temperature on various tissues of the same animal, and on the same tissue under different conditions; and again, in higher forms, the influence of the nervous system has to be considered; when, however, the organism is reduced to the lowest possible term, the single cell, these complications are greatly reduced, if not entirely eliminated.

It has been shown, however, that there are normal rhythmic fluctuations in the rate of reproduction of Paramæcium, even when the culture is subjected to the most constant environmental conditions, and that the rhythms are due to some unknown inherent factor in cell phenomena.[8] The rhythms, then, are independent of temperature. Records of a line of cells with the descending phase of a rhythm predominant, subjected to 28°, and a line of cells with the ascending phase of a rhythm predominant, subjected to 24.5°, may actually show (during the persistence of the rhythms) a more rapid rate of division at the lower than at the higher temperature (*cf.* Fig. 1). Accordingly, in this study it has been necessary to be sure that the animals subjected to the different temperatures were in comparable phases of the rhythm, or that the experiments were sufficiently prolonged to include one or more complete rhythms (*cf.* Fig. 2). It is clear that rhythms are a factor which must be taken into account in any study of the physiology of this animal.

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[5] COHEN: Vorlesung über physikalische Chemie, 1901, p. 42; HERTWIG, O.: Archiv für mikroskopische Anatomie und Entwickelungsgeschichte, 1898, li, p. 319.

[6] KANITZ: Biologisches Centralblatt, 1907, xxvii, p. 11.

[7] BOROWSKY: Archiv für Protistenkunde, 1910, xxi.

[8] WOODRUFF and BAITSELL: Journal of experimental zoology, 1911, xi, pp. 135 and 339.

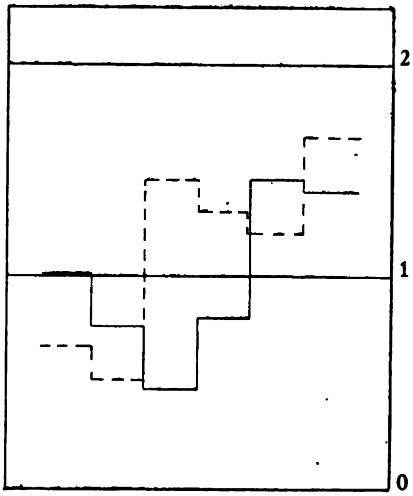

FIGURE 1. — The average daily rate of division of eight lines of Paramæcium aurelia again averaged for five-day periods at 28° C. (——— line) and 24.5° C. (- - - - line). To illustrate a case in which the rhythms of the division rate of the lines at the different temperatures alternate.

The animals used in this study were taken from the pedigree culture (I) of Paramæcium aurelia which has been under daily observation by one of us [9] for fifty-one months, and which has attained 2540 generations (up to the present time, September 1, 1911) under the conditions of a varied environment, without conjugation or artificial stimulation. A subculture (IB) was isolated from the main culture on October 1, 1910, and put on a constant culture medium of beef extract, and from this subculture other cultures were isolated which were subjected to different constant temperatures. Emphasis is placed on the fact that this culture afforded cells whose physiological and morphological condition had been studied for more than four years, and which had been subjected to a constant culture medium for ten months. All the experiments, therefore, were made on the "same protoplasm."

[9] WOODRUFF, L. L.: Biological bulletin, 1909, xvii, p. 287; Archiv für Protistenkunde, 1911, xxi, p. 263.

The paramæcia were placed in five drops of the beef extract solution on depression slides, which were kept in small moist chambers to prevent evaporation of the medium. The culture fluid consisted of a 0.025 per cent solution of Liebig's extract of beef. Sufficient culture medium was made up at the beginning of the work to last to the end. This was placed in about 100 test tubes, plugged and sterilized. The medium remained sterile until used, and consequently the culture fluid was constant throughout the experiments. Paramæcia depend upon bacteria for food, and a sufficient quantity was "automatically" transferred with the animals at the daily isolation to afford ample food until the next isolation. Since all the paramæcia used in the work were "sister" cells derived from the same culture medium, and since cross infections occurred daily, it is believed that the quality of the bacterial flora was practically the same on all the slides, and observation showed that the quantity of the bacteria at all temperatures was amply sufficient for the needs of the animals.

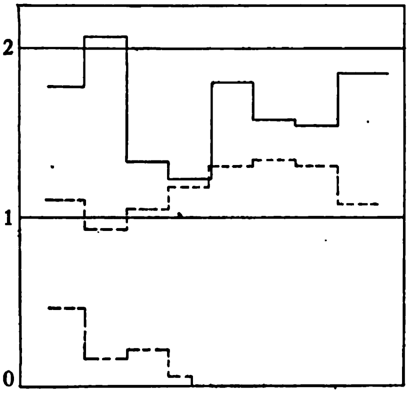

FIGURE 2. — The average daily rate of division of eight lines of Paramæcium aurelia, again averaged for five-day periods, at 28° C. (——— line), 24.5° C. (- - - - line) and 16° C. (-------- line). The eight lines at 28° and those at 24.5° were discontinued at the end of forty days. Those at 16° died out. Note that the lines at 28° during the first and last five-day periods of the experiment divided at practically the same rate. The same is true for the lines at 24.5°.

The moist chambers with the contained slides were placed in a Panum thermostat, which comprised nine compartments ranging in temperature, as adjusted during the experiments, from 35° C. to 8° C. The temperature was recorded in each compartment by a tube thermometer, a maximum and minimum registering thermometer, and in one chamber also by a thermograph. Since the method of controlling the temperature consisted of a gas flame, with an automatic regulator, at one end and a large ice chamber at the other end, the temperatures of the various compartments were not only kept practically constant, but, which is more important from the standpoint of these experiments, the very slight variations which occurred, appeared practically the same in all the compartments simultaneously. Therefore the difference between the temperatures of the various compartments was the same throughout the work. The organisms were removed from the thermostat for not more than three minutes when the daily isolations and counts were being made; the animals being transferred to fresh culture medium which had been placed the day before in the proper compartment of the thermostat, and which, consequently, was of the proper temperature. At the time of isolation a record was made of the number of divisions in each line of cells during the previous twenty-four hours, and these data form the basis of the present paper. It is believed that the conditions of the experiments in regard to ancestry of the cells, constancy of the environment, etc., were as near ideal as it is possible to make them.

Optimum Temperature. — The first point to determine was the general effect of various temperatures on the protoplasm of this pedigree culture, and accordingly subcultures, comprising eight lines of "sister" cells, were subjected to the following temperatures for forty days or until the temperature proved fatal: 8°, 16°, 21°, 24.5°, 28°, and 32° C. The results are shown below:

8°, cells dead in ± 1 day.
16°, cells dead in ± 15 days.
21°, cells dead in ± 20 days.
24.5°, cells alive and healthy at end of 40 days.
28°, cells alive and healthy at end of 40 days.
32°, cells dead in ± 2 days.

These results were clear cut, and other experiments substantiated them. It is therefore apparent that this culture of Paramæcium aurelia is adapted to a constant temperature below 32° C. and above 21° C. A careful study of the daily records shows clearly that the optimum temperature zone is between 24° and 28.5° C. The graph of the average daily rate of division, at 28°, 24.5°, and 16°, of the eight lines of cells of each series in this experiment, again averaged for five-day periods, is shown in Fig. 2.

From the studies of Schurmayer,[10] Mendelssohn,[11] Rautmann,[12]

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[10] Schurmayer: Jenaische Zeitschrift, 1890, xxiv, p. 402.

[11] Mendelssohn: Journal de physiologie et de pathologie générale, 1902, iv, p. 393.

[12] Rautmann: Archiv für Zellforschung, 1909, iii, p. 44.

Popoff,[13] and others, it appears that paramæcia will divide at temperatures between 5° and 35° C. and that the optimum temperature is between 24° and 28° C. The present data clearly show practically the same optimum for this culture, and they further show that temperatures below 21.5° and above 31.5° are sooner or later fatal. It is difficult to compare fairly these data with those derived by other workers with paramæcia because the methods used in the various experiments are so diverse, and most of the results are for effects of temporary subjection to a given temperature. It is, of course, well known that the length of time an organism is subjected to a certain temperature is an important factor in determining its effect on the animal.

TABLE I.

Daily Record of Generations of Line One (of the Eight Lines) at 28° C. and Line
The Lines were begun with Sister Cells of the Twenty-three Hundred and
in keeping the Records

June	8	9	10	11	12	13	14	15	16	17
28°	2335	2337	2338	2338	2340	2341	2343	2345	2347	2348
24.5°	2335	2336	2338	2338	2338	2338	2339	2340	2341	2341

June	29	30	July 1	2	3	4	5	6	7	8
28°	2361	2363	2366	2368	2371	2373	2375	2377	2379	2381
24.5°	2349	2351	2352	2353	2354	2356	2358	2359	2360	2362

Temperature coefficient. — A study of the daily records shows that the eight lines of "sister" cells at 28° C. for forty days gave a total of 527 divisions, while the eight lines of "sister" cells at 24.5° C. for forty days gave a total of 370 divisions. Employing the extra- and interpolation logarithmic formula used by Snyder, Rogers, and others, which takes the form

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[13] Popoff: Archiv für Zellforschung, 1909, iii, p. 124.

$$Q_{10} = \left(\frac{K_1}{K_0}\right)^{\frac{10}{T_1 - T_0}}$$

in which Q_{10} is the coefficient of the increase in reaction velocity for a rise of 10° C., and the symbols K_1 and K_0 represent constants observed (in this case cell divisions) at the temperatures T_1 and T_0 respectively, stated in degrees Celsius, it is found that the temperature coefficient derived from the above experiments is 2.74. Again, if the coefficient is computed for the rate of division of, for example, lines 1, 2, 3, and 4 (of the eight lines) at 28°, and of lines 1, 2, 3, and 4 (of the eight lines) at 24.5°, it is found to be 2.71.[14] Likewise, Q_{10} for line 1 only is 3.04. (*Cf.* Table I.) Obviously these coefficients are in remarkable agreement with the demands of van't Hoff's law.

TABLE I.

One (of the Eight Lines) at 24.5° C. for the Forty Days of the Experiment. Thirty-fifth Generation on June 8. To illustrate the Method employed of the Experiments, etc.

18	19	20	21	22	23	24	25	26	27	28
2350	2351	2351	2351	2352	2352	2353	2353	2354	2356	2358
2341	2341	2341	2341	2341	2341	2342	2342	2344	2345	2347

9	10	11	12	13	14	15	16	17	18	..
2382	2384	2386	2388	2390	2392	2394	2396	2398	2400	....
2364	2365	2367	2369	2372	2373	2375	2377	2378	2379	....

In the experiments described, a comparatively limited number of lines of cells were tested for a considerable length of time at temperatures within the *optimum zone* of the organisms employed. It seemed advisable also to determine the effects of different temperatures on a large number of lines of cells for a shorter period of time. This not only afforded, in a sense, a reciprocal experiment, but also allowed the organisms to be tested at temperatures 7° apart instead of only 3.5° apart, because, for a short period as five days, a normal rate of division could be obtained at 21° C. Accordingly one hundred lines of cells were subjected to 28° C. for five days and a similar number to 21° C. for five days. The one hundred lines at 28° gave a total of 662 divisions, or an average daily rate per cell of 1.324 divisions, while the one hundred lines at 21° gave a total of 336 divisions, or an average daily rate per cell of 0.672 of a division. Computation of Q_{10} from these data by the formula previously employed gives 2.63, which again is clearly in remarkable agreement with van't Hoff's rule.

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[14] *Cf.* Woodruff and Baitsell: Journal of experimental zoölogy, 1911, xi, No. 4, Fig. 12, for details of this phase of the experiment.

In order to test the coefficient at a lower range of temperature, three series of eight lines of cells each were carried for five days at 28°, 24.5° and 16° C. respectively. It was necessary to make the period short because the organisms were not long able to endure 16°, and beyond five days the rate of division was obviously abnormal because the cells showed a high mortality. The records of this experiment show that 71 divisions occurred in the eight lines at 28° for five days, 44 divisions at 24.5°, and 18 divisions at 16°. Q_{10} for the division rates at 28° and 16° is 3.13, and at 24.5° and 16° is 2.86. Again both of these coefficients conform closely to theoretical demands, and the fact that they are slightly higher than the average coefficient for the data given above is in accord with the results of experiments on other physiological processes — it being quite well established that the rate of acceleration decreases as one proceeds upward from 0° toward the optimum temperature.

The data which have been presented may be summarized as follows:

TABLE II.

	T_1	T_0	K_1	K_0	Q_{10}
Eight lines of cells for forty days	28	24.5	527	370	2.74
One hundred lines of cells for five days	28	21.0	662	336	2.63
Eight lines of cells for five days	28	16.0	71	18	3.13
Eight lines of cells for five days	24.5	16.0	44	18	2.86

Taking the average of the coefficients as they appear in the table, $Q_{10} = 2.84$. If, however, the coefficients are weighted on the basis of the rate of division of one cell for five days, the most fair coefficient for the entire series of experiments is obtained, $Q_{10} = 2.70$. The obvious conclusion is that the rate of cell division of Paramæcium is influenced by temperature at a velocity similar to that for a chemical reaction.

SUMMARY.

1. The optimum temperature for this culture of Paramæcium aurelia is between 24° and 28.5° C. Continued subjection to temperatures below 21.5° and above 31.5° C. is sooner or later fatal.

2. The temperature coefficient of the average rate of reproduction is approximately 2.70, and therefore the rate of cell division is influenced by temperature at a velocity similar to that for a chemical reaction.

NOTE UPON THE PREPARATION OF THROMBOPLASTIC EXTRACTS (THROMBOPLASTIN) FROM TISSUES.

By H. L. CECIL.

[From the Physiological Laboratory of the Johns Hopkins University.]

IT is well known that the aqueous or saline extracts of many tissues have an accelerating effect upon the coagulation of blood, and indeed in the blood of the vertebrates lower than the mammal the prompt clotting of the blood is dependent upon a substance present in the external tissues. The effect of these tissue extracts is shown most strikingly by their influence upon so-called peptone plasma. Plasmas of this latter sort are clotted with some difficulty by the addition of serum or of preparations of thrombin, but coagulate promptly when mixed with an extract of the tissues. In the experiments recorded in this paper peptone plasmas prepared from dogs were used exclusively to test the efficiency of the tissue extracts, and a word may be said in regard to the method of obtaining such plasmas.

Preparation of peptone plasma. — In the earlier experiments the usual method of preparing these plasmas was followed; that is to say, a 6 or 7 per cent solution of Witte's peptone was made by boiling with a 0.9 per cent solution of sodium chloride. After filtering and cooling this solution was injected under pressure into the femoral artery in amounts to give at least 0.3 gm. of the peptone to each kilogram of animal. The dogs in all cases had fasted for twenty-four hours. The animal was bled within fifteen to twenty minutes and the blood was centrifugalized at once. Following this method, the author found, as others have doubtless found before, that while some specimens of Witte's peptone gave excellent results others were almost entirely worthless, since they delayed the coagulation of the blood for only a short time. This result is easily understood when one recalls that Pick and Spiro[1] have shown that the active material of Witte's peptone is not the peptone nor the proteoses present, but an unknown substance for which they proposed the name peptozym. This peptozym in fact is to be regarded as an impurity in the Witte's peptone, and it is to be expected therefore that some preparations will contain more of it than others. After many unsuccessful preparations of peptone plasma made with the specimens of Witte's peptone which were obtainable, the author, following a suggestion contained in the work of Pick and Spiro, devised another method of obtaining an active peptozym, as follows: A large quantity of pig's fibrin obtained from the slaughter-house was first washed in running water until free from hemoglobin. It was then digested at 38° C. for several days in a large bulk of hydrochloric acid, 0.4 per cent. The solution was strained through cheese-cloth, boiled, filtered and evaporated to dryness over a water-bath. The residue was powdered and was dissolved in a small quantity of boiling water. This solution was neutralized with sodium carbonate, filtered and again evaporated to dryness over a water-bath. The residue thus obtained was used for injection. It was used in the proportion of 0.3 gm. to 1 kgm. of animal. The solution injected was made by boiling the required amount in a 0.9 per cent solution of sodium chloride and filtering. With this preparation excellent plasmas were obtained from dogs. After centrifugalizing the blood the very clear plasmas were entirely free from hemoglobin, and usually showed an entire loss of the power of spontaneous coagulation. Moreover they were entirely unaffected by mechanical particles, such as dust, chalk powder, freshly precipitated calcium oxalate, or by the addition of solutions of calcium chloride. On the other hand, addition of a suitable amount of a saline extract of muscle or of spleen caused the formation of a firm clot within five to ten minutes. Equal amounts of milk, serum, oxalated plasma, egg albumin, suspensions of red corpuscles, etc., were ineffective. According to my experience effective tissue extracts may be obtained from muscle, liver, lungs, spleen, thymus gland, lymph glands, and salivary glands. Extracts from the pancreas and intestine were inactive.

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[1] Pick and Spiro: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 235.

Isolation of thromboplastin. — Many attempts were made to isolate the active substance in these tissue extracts in some degree of purity. While these efforts were not entirely successful, they have yielded some results which it is hoped will be of value for further work. Efforts to separate the active substance of the extracts by means of fractional precipitation with ammonium sulphate gave uncertain and variable results, so that the method was abandoned. By far the most successful results were obtained with glycerine extracts of the tissues. These glycerine extracts have the advantage over the saline extracts that they are apparently permanent and a good preparation can therefore be kept for a series of experiments lasting for a long period. The method finally adopted for preparing the glycerine extracts was as follows: The organ used was either the spleen or the thymus, preferably the latter. By irrigating saline solution (NaCl 0.9 per cent) through the blood vessels the organ was first washed free of its contained blood. It was then left at room temperature for twenty-four hours, since the perfectly fresh organ was found to yield less of the thromboplastin than one which had been kept for some hours. The organ was then cut into small pieces and ground in a mortar with clean sand. The thin paste thus obtained was spread upon a glass plate and thoroughly dried in a current of warm air. The dry material was then powdered in a mortar and rubbed up for a few minutes with glycerine, and this mixture was quickly centrifugalized and the more or less clear glycerine extract was decanted from the residue. Experience showed that a rapid extraction with glycerine gave better results than a prolonged extraction. In the latter case some substances apparently passed into solution which had an inhibiting influence upon the coagulation process. In using the glycerine extract to clot a peptone plasma it was found advisable to dilute it first with two to three times its volume of water. The dilution caused the formation of a fine emulsion or granular precipitate which on standing slowly settled to the bottom. When an equal volume of this fine emulsion was added to a specimen of peptone plasma, the latter clotted usually within a few minutes. If the diluted glycerine extract was allowed to settle, the supernatant liquid was found to exhibit but little if any thromboplastic action; evidently therefore the active substance, the thromboplastin, was contained chiefly in the granular precipitate produced by the dilution. Following out the suggestion offered by this observation, an effort was made to ascertain whether the thromboplastin could be dissolved out from the granular precipitate. For this purpose some of the original glycerine extract was diluted, in centrifugal tubes, with three times its volume of water, and the solutions were then centrifugalized. The supernatant liquid was decanted, and the residue was again stirred up in water equal in amount to the first mixture. This solution was again centrifugalized, the supernatant liquid was decanted, and the residue was again treated in the same way. After several washings of this kind it was found that the precipitate had lost its thromboplastic power.

The washings, except that from the first centrifugalization which contained much glycerine, were evaporated to dryness in a current of warm air. The residue thus obtained was very small indeed in amount, but when stirred up in water it exhibited a distinct thromboplastic action upon specimens of peptone-plasma. The residue seemed for the most part to be insoluble in the water, but the solution after filtration still exerted a thromboplastic effect. It would appear from these experiments that the thromboplastin thrown down with the granular precipitate on dilution with water can be slowly washed out by repeated extraction with relatively large volumes of water. The amount of thromboplastin obtained in this way in the washings, even when these latter were evaporated to dryness, was apparently much less than that contained in the original glycerine extract, and it would seem therefore that a portion of it had undergone some change during the process. As stated above, the residue obtained from the evaporated washings was very small, too small in fact to make any satisfactory qualitative tests as to its nature.

Efforts were made to wash out the thromboplastin with other solutions, such as sodium chloride, 0.9 per cent, and sodium bicarbonate, 0.1 to 0.2 per cent, but the results were less satisfactory than with water. The main positive result therefore of the work so far reported is the discovery of a method of preparing active and permanent glycerine extracts of the thromboplastin contained in the tissues. These glycerine extracts can be kept in the laboratory for constant use.

THE INTRAVENOUS INJECTION OF THROMBIN.

BY DANIEL DAVIS.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

IN connection with the method of preparing thrombin described by Howell[1] it was thought desirable to test the effect of injecting this material directly into the circulation of the living animal. Howell describes one such experiment in his paper in which somewhat negative results were obtained, but the amounts injected were small, and in view of a possible therapeutical use of thrombin it was desirable to investigate its effect more thoroughly. Numerous observers (Schmidt, Köhler, Edelberg, *et al.*) have tested the effect of such injections, making use, however, of blood serum or impure solutions of thrombin. It would appear from their experiments that considerable quantities of thrombin may be introduced into the circulation without causing intravascular clotting, but when the amount is sufficient (Edelberg) extensive thromboses may result. In the experiments described in this note pure thrombin was employed. It was dissolved in solutions of sodium chloride (0.9 per cent) or in water according as the dry thrombin was or was not free from sodium chloride. The animals used were one rabbit, one dog, and two cats. They were anesthetized with ether and were connected with a kymographion to record blood pressure and respirations. The thrombin solutions were introduced into the jugular vein from a small burette connected with the vein by a cannula. In addition to the observations made upon the blood pressure and respirations a record was kept of the effect of the injections on the time of coagulation and the bleeding time. For the time of coagulation specimens of blood, about 2 or 3 c.c., were taken directly from the femoral artery through a cannula and tube. This blood was received into clean test tubes, and the time of coagulation was taken as the time between the reception of the blood and its setting into such a firm clot that the tube could be inverted with safety.

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[1] HOWELL: This journal, 1910, xxvi, p. 453.

The bleeding time was determined by the method of Duke.[2] That is to say, a small cut was made with scissors on the edge of the ear and the drop of blood that welled out was sopped up with filter paper. The filter paper was applied every half-minute and the bleeding-time was determined by the number and size of the drops received upon the paper. It is obvious that the method is not exact, since it is not possible to make the cuts exactly the same each time. But in a general way the results, although not entirely uniform, are distinct and satisfactory when there is any decided difference in the coagulability of the blood.

As stated above, it was possible to make but four experiments owing to the small supply of pure thrombin available. In the first, rabbit, the thrombin was injected in amounts equal to 28 mgm. per kilogram of body-weight; in the second, dog, the preparation was 28 mgm. of thrombin per kilogram of weight; in the third, cat, 20 mgm. per kilogram, and in the fourth, cat, 56 mgm. per kilogram. It should be stated that the quantitative estimation of the amount of thrombin was made by taking a known volume of solution of pure thrombin and evaporating to dryness in a weighed platinum crucible. The residue was heated to constant weight at 110° C. and was subsequently incinerated. The difference between the weight of the dried residue and the incinerated residue was taken as the weight of thrombin in the given volume of solution. So far as the kymographic record is concerned the results were substantially entirely negative — there was no decisive effect upon pulse rate, blood pressure, or respirations. In regard to the coagulation time and bleeding time the results obtained may be seen from the following brief summary:

Experiment 1. — Rabbit. Thrombin injected in the proportion of 28 mgm. per kilogram of animal.

Before the injection, bleeding time, four drops (two minutes).

Sixteen to twenty minutes after the injection	Coagulation time, fourteen minutes. Bleeding time, ten drops (five minutes).
Thirty to thirty-five minutes after the injection	Coagulation time, five minutes. Bleeding time, ten drops (five minutes).
Fifty minutes after the injection.	Coagulation time, four minutes. Bleeding time, not taken.

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[2] Duke: Journal of the American Medical Association, 1910, lv, p. 1185.

Experiment 2. — Dog. Thrombin injected in the proportion of 28 mgm. per kilogram of animal. Before the injection, bleeding time, nine drops (four and one-half minutes).

Twelve minutes after the injection	Coagulation time, five minutes. Bleeding time, seven drops (three and one-half minutes).
Twenty-eight minutes after the injection	Coagulation time, ten minutes. Bleeding time, twenty drops (ten minutes).
Forty-three minutes after the injection	Coagulation time, eleven minutes. Bleeding time, thirteen drops (six and one-half minutes).
Sixty-nine minutes after the injection	Coagulation time, five minutes. Bleeding time, twenty-eight drops (fourteen minutes).

Experiment 3. — Cat. Thrombin injected in the proportion of 20 mgm. per kilogram of animal. Before injection, bleeding time, six drops (three minutes).

Fifteen minutes after the injection	Coagulation time, twelve and one-half min. Bleeding time, fourteen drops (seven min.).
Thirty-four minutes after the injection	Coagulation time, two minutes. Bleeding time, fourteen drops (seven min.).
Fifty minutes after the injection	Coagulation time, three minutes. Bleeding time, eight drops (four minutes).

Experiment 4. — Cat. Thrombin injected in proportion of 56 mgm. per kilogram of animal. Before injection, bleeding time, three drops (one and one-half minutes).

Seven and one-half minutes after the injection	Coagulation time, sixteen and one-half min. Bleeding time, seven drops (three and one-half minutes).
Twenty-one minutes after the injection	Coagulation time, twelve minutes. Bleeding time, four drops (two minutes).
Forty-two minutes after the injection	Coagulation time, seven minutes. Bleeding time, six drops (three minutes).
Sixty-two to seventy minutes after the injection	Coagulation time, seven minutes. Bleeding time, four drops (two minutes).

Owing to the small number of experiments which it was possible to make, it is not advisable to draw any conclusions in regard to the difference in reaction among the several kinds of animals used. In general, however, it may be stated with confidence that the injection of pure thrombin into the circulation, within the limits of 20 to 56 mgm. per kilogram of animal, does not cause intravascular clotting. On the contrary, the coagulability of the blood is diminished for a certain short period of time.

The diminished coagulability, in the experiments made, appeared within seven to twenty-eight minutes and lasted from thirty minutes to an hour or more, but was clearly shown in each animal. It does not seem possible to explain this effect otherwise than by assuming that the injection of thrombin had called forth a production of antithrombin. It is well known that intravascular injections of so-called peptone solutions produce in the dog a rapid formation of antithrombin within the animal's body. It is also known that the substance calling forth this reaction is not the peptone or propeptone in the solution, but some probably simpler body of unknown nature which has been named peptozym by Pick and Spiro. The work of Delezenne, Nolf, and others would indicate that the action of peptozym is indirect, that through some effect upon the leucocytes substances are produced which stimulate the liver to form antithrombin. However this may be, the point of interest in connection with this paper is that it has been denied (Nolf[3]) that thrombin injected directly into the circulation can itself arouse a production of antithrombin. If the latter substance is, as it seems to be, a genuine antibody to the thrombin, then, according to all of our analogies with similar bodies, the presence of thrombin itself should lead to its formation. That this is the case seems to be demonstrated by the experiments given above. A noteworthy fact in regard to this reaction is the great promptness with which it occurs. The rapidity with which a new formation or output of antithrombin follows upon the injection of peptozym or of an excess of thrombin into the circulation suggests naturally that this mechanism may come into play normally to protect the body from intravascular clotting. In an accompanying paper (Howell) the view is proposed that the normal fluidity of the blood is maintained by the constant presence of antithrombin in slight excess. The fact that an excess of thrombin acts quickly as a stimulus to the tissues producing antithrombin suggests a means by which under normal conditions a balance in favor of the antithrombin is maintained in the blood.

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[3] NOLF: Archivio di fisiologia, 1909, vii, p. 1.

FURTHER STUDIES ON THE RELATION OF THE PANCREAS TO THE SERUM AND LYMPH DIASTASES.

By L. K. GOULD and A. J. CARLSON.

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THE previous work reported from this laboratory has not supported the view that the blood and lymph diastase is pancreatic amylopsin absorbed directly from the pancreas or from the intestines.[1] But the great diminution in the diastatic power of the blood following pancreatectomy has not been explained. In the present study we have sought further light on this question by determining the influence on the serum diastase of (1) atrophy of the pancreas parenchyma, (2) extirpation of the atrophied pancreas. The hypothesis finally advanced that the concentration of the serum diastase in so far as the pancreas is concerned is mainly determined indirectly by the internal secretion of the pancreas, was not the starting-point but grew out of the experimental results.

The status of the problem may be briefly summarized as follows:

1. **The identity and multiplicity of the serum diastases.** — Bial's[2] view that the serum diastase differs from ptyalin and amylopsin may be correct, but the experimental results in support of it were shown to be erroneous by Hamburger.[3] Evidence tending to show that there are a number of diastatic ferments in serum and lymph has been put forth by Hamburger, Borchardt,[4] Ascoli and Bonfanti.[5] The last two experimenters claim to have developed specific serum

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[1] CARLSON and LUCKHARDT: This journal, 1908, xxiii, p. 148; OTTEN and GALLOWAY: *Ibid.*, 1910, xxvi, p. 347.

[2] BIAL: Archiv für die gesammte Physiologie, 1892, lii, p. 137; liii, p. 157.

[3] HAMBURGER: *Ibid.*, 1896, lx, p. 543.

[4] BORCHARDT: *Ibid.*, 1904, c, p. 259.

[5] ASCOLI and BONFANTI: Zeitschrift für physiologische Chemie, 1904, xliii, p. 156; Münchener medizinische Wochenschrift, 1904, li, p. 1466.

diastases by feeding different kinds of carbohydrates, but this could not be substantiated by later workers.[6]

2. **Serum diastases and tissue diastases.** — A number of workers have published results tending to show that the serum diastases come from the formed elements in the blood, especially the leucocytes.[7] The identity of the liver and the serum diastases is accepted by many investigators, but the question whether the liver cells absorb the diastase from the serum or secrete the diastase into the serum is still undecided.[8]

3. **Ligation of the pancreatic ducts and extirpation of the pancreas.** — Most observers agree that duct ligation increases the serum diastases by direct absorption of amylopsin from the pancreas.[9] But no effect from this operation has been reported for the pigeon,[10] and an actual decrease in the serum diastase for the rabbit.[11] Pancreatectomy leads to a decrease in the diastases.[12]

4. **Diastase concentration of the venous blood from different organs.** — The literature on this point is very contradictory. An excess of

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[6] Wohlgemuth: Verhandlungen des Kongresses für innere Medizin, 1908, xxv, p. 500; Carlson and Luckhardt: This journal, 1908, xxiii, p. 148.

[7] Tiegel and Plasz: Archiv für die gesammte Physiologie, 1872, vi, p. 24; vii, p. 391; Castellimo and Paracca: Archives italiennes de biologie, 1895, xxiii, p. 372; Berestnew: Archives de la Société de Biologie de St. Petersbourg, 1895, iii, p. 40; Acholme: Comptes rendus de la Société de Biologie, 1899, lx, p. 568; Haberlandt: Archiv für die gesammte Physiologie, 1910, cxxxii, p. 175.

[8] Bial: Archiv für die gesammte Physiologie, 1894, lv, p. 434; Borchardt: *Ibid.*, 1904, c, p. 259; Pugliese: Archives italiennes de biologie, 1907, xlvi, p. 1; Manzini: Archiv di farmacologia sperimentale, 1905, iv, p. 120; Macleod and Pearce: This journal, 1910, xxv, p. 255.

[9] Lepine and Barral: Comptes rendus de la Société de Biologie, 1891, cxiii, p. 729; Schlesinger: Verhandlungen des Kongresses für innere Medizin, 1908, xxv, p. 505; Wohlgemuth, *Ibid.*, p. 500.

[10] Langendorff: Archiv für Physiologie, 1879, p. 1.

[11] Loeper and Fiaci: Comptes rendus de la Société de Biologie, 1907, lxiii, p. 266; Clerc and Loeper, *Ibid.*, lxvi, p. 871.

[12] Lepine and Barral: Revue de Médecine, 1892, p. 488; Kaufmann: Comptes rendus de la Société de Biologie, 1895, xlvi, p. 130; Bainbridge and Beddard: Biochemical journal, 1907, ii, p. 89; Schlesinger: Deutsche mediziniche Wochenschrift, 1908, xxxiv, p. 593; Wohlgemuth, Biochemische Zeitschrift, 1909, xxi, p. 381; Otten and Galloway: This journal, 1910, xxvi, p. 347.

diastase has been reported for the blood of the pancreatic vein.[13] Other observers have been unable to detect any difference in diastase concentration in the blood from the different organs.[14]

METHODS.

The dogs were bled from the external saphenous vein, but in the cases of pancreatectomy where the animals lived for several weeks after the operation, the later bleedings were sometimes directly from the heart. The pigs were bled from a cut in the ear or the tail. The blood was defibrinated and centrifuged for an hour and a half.

Salkowski's method was used for the determination of the concentration of the diastases. One c.c. of the serum was added to 20 c.c. of a 1 per cent solution of soluble starch (Merck), the mixture incubated at 38° C. and tested at intervals with a very weak solution of I in KI. The complete disappearance of the iodine blue reaction was taken as the end point. Duplicates were used in all cases. They were found to agree, thus indicating the reliability of the method.

RESULTS.

1. Ligation of the pancreatic ducts, and partial atrophy of the pancreas in the dog. — As has been previously reported, it was found that the daily diastatic power exhibited by the serum of normal animals of the same species varied within quite narrow limits. But in a series of experiments continued for seven or eight consecutive days, it is not uncommon to find a variation within the range of twenty to thirty minutes, and in the case of one dog, where observations were made daily for nineteen days, as great a variation as one hour and twenty minutes or 100 per cent was observed. Such a great variation in only one case out of fifteen certainly does not indicate a general characteristic of the species. That there should be some variation from day to day, in the diastatic power of the serum, however, is to be expected, especially if the ferment is a product of cellular activity in general. A few comparative observations on the diastatic power of the serum of different species may be noted in this connection. Thus under the same conditions, the digestion time to the erythrodextrin stage in the case of serum of the duck is eight minutes, in the case of dogs the average is about two hours, and with goats' and sheep's serum there is still a deep blue color with iodine at the end of twenty-four hours.

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[13] CAVAZZANI: Archives italiennes de biologie, 1894, xx, p. 241; SCHLESINGER, Deutsche medizinische Wochenschrift, 1908, xxxiv, p. 593.

[14] WOHLGEMUTH: Biochemische Zeitschrift, 1909, xxi, p. 381; CARLSON and LUCKHARDT: This journal, 1908, xxiii, p. 148; MACLEOD and PEARCE: *Ibid.*, 1910, xxv, p. 255.

In every case after the ligation of both pancreatic ducts as well as after the complete ligation of the pancreatic tissue along the duodenum, there was a great increase in the diastatic power of the blood serum. The maximum increase is usually not reached until forty-eight to seventy-two hours after the ligation. It remains at that concentration for a period of three to seven days and then gradually decreases, so that in ten to fifteen days following the operation the diastatic power has returned, as a rule, to normal. In two cases the drop following the initial rise did not quite reach the normal level.

In eight cases out of eleven there was observed a very pronounced secondary rise about the third or fourth week. In several cases this increase in diastase concentration was as marked as that following immediately upon the ligation. Dogs I and VII showed also a third rise about the fifth week. The length of time required to return toward the normal after the secondary rise is usually much greater in the dogs in which total ligation had been performed than in the case of those in which the two ducts only are ligated, and in three cases out of four even at the end of two months and a half the diastatic power of the serum still remained considerably above the normal. Details of some of the typical experiments are given in Table I.

If the pancreas is the main source of the blood diastases, it seems reasonable to expect that with the degeneration of the parenchyma there would be a corresponding decrease in diastatic power of the serum. Although in every case of our ligations there was considerable atrophy of the pancreas, and especially so in the case of Dog V, where the pancreas on autopsy was found to be only about one fifth the normal size, yet there had been in each case of mere ligation of the ducts or all the pancreatic-duodenal connections, a sufficient re-establishment with the duodenum to permit the organ to re-establish normal function at least in part.

In order to shut out completely all the pancreatic secretion from the duodenum, and to destroy as far as possible all opportunity of re-establishment of the ducts, the following operation was performed in two dogs. The middle third of the pancreas lying along the duodenum was carefully dissected away from the pancreatic-duodenal vessels and, after ligation of the head and tail, the middle third was removed. The two cut ends were then cauterized and left in their normal position with the blood supply intact. Both animals became poorly nourished and lost steadily in weight during the first month, although they did not develop diabetes. At the end of the fifth week the diastatic power of the serum still remained as great as the initial rise following ligation of the ducts in the other dogs. One of the dogs died of pneumonia at the end of six weeks. The other dog (No. XV, Table I) was kept for six months. At that time the diastase concentration was normal, despite the almost complete atrophy of the pancreas left at the time of the operation.

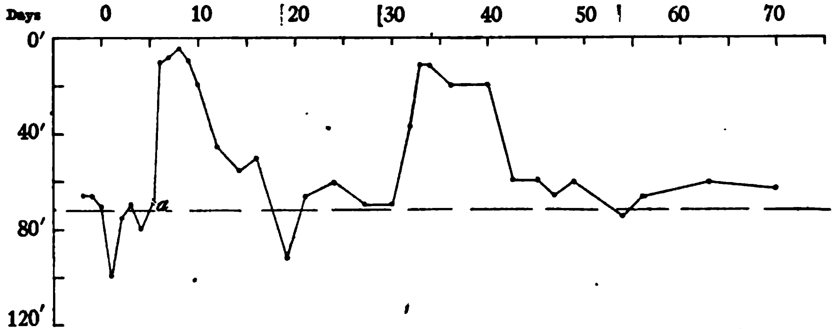

Figure 1. — Dog II. Curve of the diastatic power of serum after ligation of the two main pancreatic ducts at *a*. Note the second period of increase in the fourth and fifth weeks. Diastatic power expressed in minutes.

2. Complete removal of the normal and the partly atrophied pancreas in dogs. — The five experiments made in this series were prompted by the observations of Wohlgemuth,[14] and of Otten and Galloway in this laboratory, that following the initial decrease in diastase concentration as the result of pancreatectomy there is a gradual return towards the normal concentration. These results are difficult to explain on the view that the pancreas is the main source of the serum diastase. They also seem to run counter to our hypothesis that the diminished concentration of the serum diastase following pancrea-

TABLE I.

Detail of five Typical Experiments showing the Influence of various Methods of Ligation of the Pancreatic Ducts and varying Degrees of Pancreatic Atrophy on the Concentration of the Serum Diastase.

Dog I. Ligation of two main pancreatic ducts, Oct. 31.		Dog II. Ligation of two main pancreatic ducts, Oct. 31.		Dog VI. Ligation of pancreatic ducts. Dec. 2. Religation of same and of an additional duct, Jan. 19.		Dog VIII. Ligation of all pancreatic-duodenal connections, Jan. 12.		Dog XV. Extirpation of middle third of pancreas adjacent to duodenum with ligation and electric cautery of cut end of the head and the tail of the gland.	
Date.	Digestion time.	Date.	Digestion time.	Date.	Digestion time.	Date.	Digestion time.	Date.	Digestion time.
Oct. 24	hrs. 1.50	Oct. 24	hrs. 1.05	Nov. 28	hrs. 2.25	Jan. 9	hrs. 2.10	Apr. 13	hrs. 1.35
25	2.10	25	1.05	29	2.20	10	2.00	14	Ligation, etc.
26	2.10	26	1.10	30	2.10	11	2.00	15	0.13
27	2.20	27	1.40	Dec. 1	2.00	12	Ligation	17	0.07
28	2.00	28	1.15	2	Ligation	13	0.08	21	0.06
29	2.20	29	1.10	3	1.05	14	0.05	28	0.12
30	2.05	30	1.20	5	0.45	16	0.30	May 5	0.10
31	Ligation	31	Ligation	7	0.45	18	1.20	17	0.15
Nov. 1	0.30	Nov. 1	0.10	10	0.40	20	1.35	26	0.15
2	0.25	2	0.08	14	1.00	23	1.50	June 9	0.40
3	0.20	3	0.05	19	1.40	25	1.20	Oct. 14	2.00
4	0.45	4	0.10	21	1.40	28	0.30	Dog in good condition. Killed. Autopsy showed the head of the pancreas as a hardened string of greatly atrophied tissue.	
5	0.50	5	0.20	28	1.35	30	0.12		
7	1.20	7	0.45	Jan. 4	2.00	Feb. 1	0.15		
9	1.55	9	0.55	11	1.50	3	0.15		

11	1.50	11	0.50	18	1.50	6	0.25	Fresh weight 1 1/2 gm. The tail of the pancreas could not be recognized macroscopically. There was no evidence of duct union with the duodenum.
14	1.20	14	1.35	19	Religation	8	0.45	
16	1.05	16	1.05	20	0.12	10	0.50	
19	1.20	19	1.00	21	0.08	14	1.10	
22	1.35	22	1.10	23	0.10	17	0.50	
25	1.35	25	1.10	25	0.30	21	1.30	
28	1.35	28	0.15	28	0.30	24	1.00	
Dec. 1	1.10	29	0.12	30	0.35	28	1.15	. . . .
5	1.40	Dec. 1	0.20	Feb. 1	0.55	Mar. 3	1.30	
7	1.45	5	0.20	3	0.15	9	1.30	. . . .
10	2.15	7	1.00	6	1.15	22	1.45	
12	2.15	10	1.00	8	2.15	Apr. 4	1.45	. . . .
14	1.45	12	1.05	10	2.10			
19	2.05	14	1.00	14	2.05	. . .	. . .	. . . .
21	1.55	19	1.15	17	1.40			
28	1.05	21	1.05	21	1.10	. . .	. . .	. . . .
Jan. 4	2.05	28	1.00	24	1.55			
11	2.00	Jan. 4	1.05	28	1.10	. . .	. . .	. . . .
				Mar. 3	1.55			
. . .	. . .	. . .	. . .	9	1.55	. . .	. . .	. . . .
				22	1.40			
. . .	. . .	. . .	. . .	Apr. 4	1.30	. . .	. . .	. . . .

tectomy in dogs is an indirect effect from the loss of the internal secretion of the pancreas. The studies of Otten and Galloway did not comprise a sufficient number of dogs to settle the point definitely, especially since one of the dogs exhibited great fluctuation in the diastase concentration.

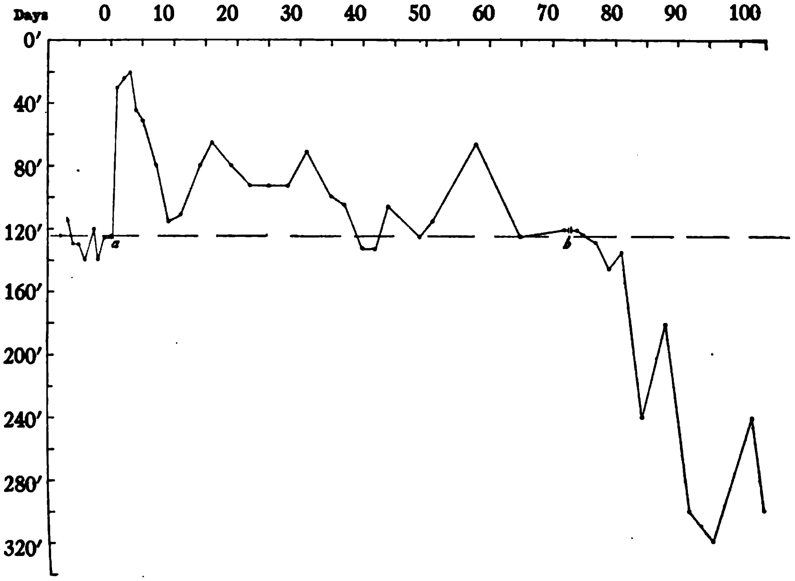

Figure 2. — Dog I. Curve of diastatic power of serum after ligation of the two main pancreatic ducts (*a*) and subsequent pancreatectomy (*b*). Note the second rise in the third week, and the third rise in the seventh week. Diastatic power expressed in minutes.

Our results are summarized in Table II. There is practically no difference in the effect on the diastatic power of the serum between the removal of a normal and a partly atrophied pancreas, except that the decrease in the diastase seems less rapid in the latter cases. More experiments are necessary to establish this point. We expect that further work will verify the present indication, because when the pancreatic ducts are ligated the organism has time for such adjustment or compensation for the interference with or loss of the external and internal secretion as may be possible, and is therefore less rapidly damaged by the great disturbance of final pancreatectomy. Moreover, there is no persistent return toward the normal diastase con-

TABLE II.

DETAIL OF FIVE EXPERIMENTS SHOWING THE INFLUENCE OF EXTIRPATION OF THE PANCREAS, NORMAL AND IN VARYING STAGES OF ATROPHY, ON THE CONCENTRATION OF THE SERUM DIASTASE.

Dog I. Ligation of pancreatic duct, Oct. 31. Pancreatectomy, Jan. 12.		Dog III. Ligation of pancreatic duct, Nov. 12. Pancreatectomy, Jan. 19.		Dog IV. Ligation of two pancreatic ducts, Oct. 31. Pancreatectomy, Jan. 12.		Dog XII. Normal pancreatectomy, Feb. 27.		Dog XIII. Normal pancreatectomy, Feb. 27.	
Date.	Digestion time.	Date.	Digestion time.	Date.	Digestion time.	Date.	Digestion time.	Date.	Digestion time.
Jan.	hrs.	Dec.	hrs.	Dec.	hrs.	Feb.	hrs.	Feb.	hrs.
4	2.05	19	2.15	28	2.00	20	1.50	20	1.50
11	2.00	21	2.05	Jan. 4	2.00	21	1.40	21	1.45
12[1]	....	28	2.10	11	2.00	23	1.35	23	1.45
13	2.00	Jan. 4	2.15	12[1]	....	25	1.45	25	1.45
14	2.05	11	2.00	13	2.30	27[1]	....	27[1]	....
16	2.10	18	2.00	14	3.00	28	3.30	28	2.30
18	2.25	19[1]	....	16	4.15	Mar. 3	3.00	Mar. 3	2.25
20	2.15	20	3.30	18	3.30	6	3.45	6	3.30
23	4.00	21	4.45	20	3.25	9	4.30	9	3.30
26	3.00	23	5.00	23	4.30	13	4.45	13	3.50
30	5.00	..	....	26	4.00	..	....	22	3.10
Feb. 3	5.30	..	....	30	4.00	..	....	Apr. 16	4.30
8	4.00	..	....	Feb. 3	6.00	..	....	..	....
17	5.00	..	....	8	5.00	..	....	..	....
..	....	..	....	17	6.00	..	....	..	....

[1] Pancreatectomy.

centration after the initial drop. The fluctuations in the diastase concentration is greater than in the normal animal, but the general direction of the curve is downward, even when the tests were continued for six weeks after the pancreatectomy. This general drop of the curve cannot be explained as due to a gradual elimination or destruction of the diastase, the source of the ferment being removed in the removal of the pancreas. It seems to us that this interpretation is rendered untenable by the very fluctuation in the diastatic power. If the diastase is a product of the liver and the tissues in general, the gradual drop of the curve is to be expected as a concomitant to gradual starvation and depression of the tissue cells in consequence of the loss of the internal secretion of the pancreas.

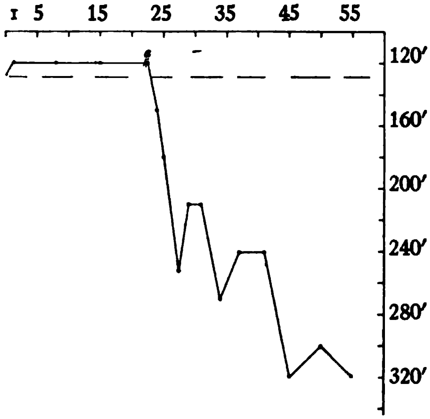

FIGURE 3. — Dog IV. Curve of diastatic power of serum following pancreatectomy. Note the general descent of the curve interrupted by periods of increased concentration. Diastatic power expressed in minutes.

It may be remarked in this connection that while the diastatic power of dog's serum four to six weeks after complete pancreatectomy is much smaller than the normal, yet it is many times greater than the diastatic power of the normal serum of many species, for example, goat and sheep.

We call special attention to Dog I, Table II. It will be noticed in this case that the diastase concentration continues practically normal for eight days after the pancreatectomy, when the usual fall appears and continues for the next four weeks. This case is unique for the dog, at least as regards the experience of our laboratory. The explanation cannot be sought in an incomplete pancreatectomy, for the dog ran the usual diabetes and weight curves, and post mortem revealed no trace of pancreatic tissue. This case seems inexplicable on the hypothesis that the serum diastase is pancreatic amylopsin, for we know that the serum diastase is constantly being lost in the urine.

3. **The influence of pancreatectomy on the concentration of the serum diastase in the pig.** — According to Minkowski, almost complete pancreatectomy[15] in the pig does not result in the severe diabetes

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[15] The extirpated portion of the pancreas weighed 81 gm., the portion left *in situ* weighed 2.5 gm. when removed from the pig thirty-one days after the first operation.

typical for other mammals.[16] The explanation of this difference has not been worked out. The tissues of the pig must either retain more primitive power enabling them to carry on their normal processes in the absence of the internal secretion of the pancreas; or there is not as well-defined specialization of organ function in the pig. The internal secretion of the pancreas, for example, might be produced both by the pancreas and by the duodenal glands. These hypotheses are now being tested in this laboratory.

Assuming that the pig's pancreas secretes the ordinary pancreatic amylopsin, it seems to us that pancreatectomy in pigs would constitute an *experimentum crucis* for or against the hypothesis that the serum diastase is absorbed pancreatic amylopsin. If this hypothesis is correct, pancreatectomy in the pig should yield a serum diastase curve similar to that in the dog. On the other hand, if our hypothesis that the concentration of the serum diastases is a function of tissue activity in general, which is in turn determined by the internal secretion of the pancreas, is a close approximation to the truth, pancreatectomy ought to have less effect in the pig than in the dog.

Our results on two pigs are given in Table III. Complete pancreatectomy in pigs is an exceedingly difficult operation, partly because of the thick abdominal wall, but mainly because of the distribution and the great softness of the pancreas itself. In consequence of this, one of the operations turned out to be incomplete, about one tenth of the pancreas being left in the mesentery. The other operations appeared to be complete, but it is possible that fragments had been left but were overlooked in the mass of connective tissue adhesions found at autopsy. The pigs exhibited little or no digestive disturbance after the first two weeks. They continued to increase in size and weight, but not so rapidly as before the operation. The urine was tested for sugar every third or fifth day for two months after the pancreatectomy, with negative results. The urine of Pig II was not tested after June 5. On June 30, the diastase concentration of the blood having returned to normal, we made an exploratory laparotomy to determine whether the pancreatectomy was complete. The stomach, intestines, liver, and mesentery formed one mass of adhesions. The pig was killed, and 25 c.c. of urine in the bladder contained 2 per cent dextrose. This urine was probably secreted during the anesthesia and the rather severe manipulation of the abdominal organs, which lasted for one and one-half hours, and hence the glycosuria.

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[16] MINKOWSKI: Archiv für experimentelle Pathologie und Pharmakologie, 1893, xxxi, p. 93.

TABLE III.

Summary of Experiments on two Pigs showing the Influence of Partial and probably Complete Pancreatectomy on the Concentration of the Serum Diastase.

Pig I.[1] Weight 6.5 K. Pancreatectomy, Mar. 30.		Pig II.[2] Weight 5 K. Pancreatectomy, Apr. 14.	
Date.	Digestion time.	Date.	Digestion time.
Mar.	hrs	Mar.	hrs.
17	1.15	13	1.30
30	Pancreatectomy	Apr. 13	1.20
Apr. 4	1.20	14	Pancreatectomy
12	1.30	17	1.30
21	1.40	21	2.30
28	2.00	28	2.30
May 10	2.15	May 10	2.15
June 5	2.00	June 30	1.30

[1] Pig killed. Autopsy disclosed a small piece of apparently normal pancreas in the dorsal. No diabetes. Gain in weight, Mar. 17 to June 5 = 10 K.

[2] Pig killed. Autopsy showed all or at least nearly all pancreas removed. No diabetes. Gain in weight, Mar. 13 to June 30 = 9 K.

The tables show that in Pig I, which retained about one tenth of its pancreas, the diastase concentration of the serum remained normal for three weeks after the operation, when a slight drop appeared. This slightly lower level was maintained till the end of the experiment.[17] In Pig II, with apparently complete pancreatectomy, the diminution of the serum diastase appeared some time between the fifth and the seventh day after the operation. At the end of four weeks there began a return to normal, and the normal concentration was restored some time before the end of the tenth week.

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[17] The experiment was terminated prematurely by the development of a large hernia in the region of the abdominal incision.

These two experiments seem significant. *If under normal conditions in the pig the pancreas constituted the main source of the serum diastase, we should have obtained a greater and a more rapid diminution of the diastase form of the blood as a result of partial or complete pancreatectomy.* The facts can readily be brought in line with the view that the internal secretion of the pancreas is an indirect fact in the maintenance of the diastase concentration in the serum. For while the tissues were not sufficiently impaired to cause diabetes, more accurate tests would probably have showed a lowered sugar tolerance, and hence some depression and consequent diminution in the diastase.

4. **The effect of prolonged fasting on the concentration of the serum diastases.** — If the serum diastase is mainly pancreatic amylopsin absorbed from the gland or from the intestines, prolonged rest of the gland ought to decrease the diastatic power of the blood inasmuch as the blood diastase is constantly being excreted in the urine, and possibly destroyed or lost in other ways. Accordingly, two dogs were kept on water for ten days, and the diastatic power of the blood during this period compared with that on normal feeding. There was no difference in the diastase concentration. These results agree with earlier observations of Wohlgemuth.

It is highly probable that the pancreas is not entirely inactive during the whole of a ten-day fasting period.[18] Pawlow has brought forth some evidence that the pancreas can be called into activity by cerebral impulses acting through the pancreatic secretory fibres in the vagi nerves (appetite secretion). And in so far as a similar mechanism may cause occasional activity of the gastric glands during fasting, the gastric juice would in turn result in activity of the pancreas through local reflexes and hormones. But there can be little doubt that there is much less external secretion produced by the pancreas during a ten-day period of fasting than during a corresponding period of normal feeding.

5. **The concentration of the diastase in exudates rich in leucocytes.** — With the injection of aleuronat suspension into one pleural cavity an inflammatory reaction is inaugurated which results in a serous

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[18] Boldireff: Archiv des sciences biologiques, 1905, xi, p. 7.

exudate swarming with leucocytes into both cavities. Twenty-four hours after such an injection the dogs were killed and the fluid from both cavities was immediately tested. Some of the fluid from each cavity was also centrifuged for an hour and a half, and in this manner a still greater concentration of leucocytes was obtained. Tests were made of fluid taken from both the top and the bottom of the centrifuge tubes. In all cases, however, the digestion time of 1 c.c. of the fluid in 20 c.c. of the 1 per cent starch solution was five to six hours, while the serum from the animals in like proportion digested the starch in one and a half to two hours.

We do not take these results to imply that the leucocytes produce no diastase. The results show, however, that the leucocytes are a less important factor in the production of serum diastase than Haberlandt's work would indicate.

6. **Discussion of the results.** — We agree with the earlier workers in ascribing the great increase in the diastatic power of the blood serum which follows ligation of the pancreatic ducts to the resorption of amylopsin directly from the gland. The rapid drop following the initial rise may be explained in three ways. In the first place, there may be an increased destruction or excretion of the ferment, for the fact that normally the concentration varies within rather narrow limits points to some regulating mechanism by means of which an equilibrium between production and elimination or destruction is maintained. This mechanism is not very sensitive or delicately adjusted, however. In some cases the fall in diastase concentration is due to diminished absorption from the gland owing to re-establishment of duct connections with the duodenum. Finally, the ligation of the ducts produces edema of the pancreas within the first twenty-four hours from pressure of the secretion in the duct system. This pressure may have an injurious action on the cells which leads in turn to diminished activity and possibly pressure atrophy. This would lead to diminished secretion and decreased pressure, and hence renewed activity of the pancreas parenchyma and a second period of edema of the gland, etc. We are inclined to interpret the second and third periods of high diastase concentration in the blood to these conditions. The final return to normal or considerably above, as in the case of complete ligation, would represent the establishment of an equilibrium between the pancreatic activity, the duct pressure,

the absorption and the excretion rate. It will be recalled that there is no decrease in serum diastase *pari passu* with the decrease in the amount of parenchyma in the dog. This was especially noteworthy in case of Dogs III, V, and XV. This fact, taken in connection with the results obtained from pancreatectomy both in the dogs and the pigs, suggests that the pancreas is not an important direct source of the blood diastases under normal conditions. While the rapid decrease in concentration of the serum diastases that usually follows pancreatectomy in dogs may be explained, as Schlesinger suggested, by the assumption that we have removed the chief source of supply, the explanation fails when applied to the results in the case of Dog I and especially in the pigs. Neither can we attribute the decrease to the progressive emaciation, for with ten days' fasting there is no change in the diastatic activity of the serum. The rapid fall in concentration observed in the four cases might also be due to a rapid destruction or elimination of the ferment, owing in part to the polyurea, but experiments in our laboratory by Dr. J. Van de Erve have convinced us that this explanation is insufficient. *It seems more probable that the decline is due mainly to a decreased production owing to a certain form of depression of all the tissue cells which results from the loss of the internal secretion of the pancreas.*[19] On this hypothesis we would expect a diminished concentration of diastase in all organs following pancreatectomy. But such a fact, if established, would not demonstrate our hypothesis, because of the difficulty or impossibility of removing all the tissue lymph from an organ.

The above generalization is advanced mainly as a working hypothesis. It is obvious that we are not yet in possession of sufficient data on this point to permit of generalization applicable to all metazoa or even to all vertebrates. The cause and significance of the great variations in the concentration of serum diastase in different species must be worked out. It is not improbable that the relative importance of the various factors that go to make up the mechanism for regulating the diastase content of the body fluids differs in different species.

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[19] If the interpretation given by Carlson and Drennan of the fact that in the dog pancreatectomy in late stages of pregnancy does not cause diabetes until the fetuses are removed from the uterus is correct, it should be possible to demonstrate the correctness or inadequacy of this hypothesis in the dog by pancreatectomy. In late pregnancy this operation should cause little or no decrease in the serum diastases.

The view previously expressed by one of the authors that the serum diastases are tissue discards and of no further importance in the body economy is strengthened by the fact that the species so far studied do not possess *efficient* mechanisms for maintaining a fairly constant diastase concentration under even slightly adverse conditions. It is otherwise with the salts, proteins, and carbohydrates of the blood, and even with the ferment lipase, as has been shown by work in this laboratory by C. L. von Hess. In the dog the enormous rise in diastase concentration represented by an increase in digestive power from one hundred and twenty minutes to five minutes seems to cause no metabolic disturbance. The tissues of the duck work normally with an extremely high diastase content in the body fluids, while the sheep and the goat live their normal lives with only a trace of serum diastases.

SUMMARY.

1. Ligation of both pancreatic ducts as well as all the pancreatic tissue along the duodenum in dogs causes a great increase in the diastatic power of the blood serum, within twenty-four hours. This is probably due to absorption of amylopsin direct from the gland. The initial rise is followed by a return towards the normal, and this again followed by a secondary rise which may be as marked as the first. There may also be a third rise. An explanation of this remarkable rhythm is essayed in the discussion.
2. The time required to return to near the normal level is much greater in the case of complete ligation than in the cases of partial ligation.
3. A considerable atrophy of the pancreas parenchyma is not followed by a corresponding decrease in the diastatic power of the blood serum. Removal of the atrophied pancreas results in a decreased diastatic power of the serum.
4. Extirpation of the pancreas in dogs generally causes a rapid decrease in the concentration of the blood diastases, but this decline may not appear until as late as the eighth day following the operation. There are great fluctuations, but no distinctly continuous return

towards the normal diastase concentration. The diminished concentration is probably due in the main to decreased production.

5. Almost complete pancreatectomy in pigs does not cause a decline in diastatic activity of the serum until a considerable period after the operation, and the depression when it does appear is slight. The new level thus established tends to remain constant or returns to normal.

6. Serous exudates very rich in leucocytes, obtained by the injection of aleuronat into the pleural cavity, have much less diastatic power than the serum from the same animal.

7. These experiments seem to point to the conclusion that the pancreas or the leucocytes are not the main or the important seat of production of the blood diastases. The hypothesis is advanced that the relation of the pancreas to the serum and lymph diastases is primarily an indirect one exercised through the medium of its internal secretion, and the decrease in diastatic power following removal of the pancreas is due to a certain depression of all the tissue cells, particularly the liver, which results in the decreased output of the diastase into the body fluids.

ON THE RÔLE OF THE KIDNEYS IN THE REGULATION OF THE CONCENTRATION OF THE SERUM DIASTASE.

By J. Van de ERVE.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

Introduction.

IT is well established that complete pancreatectomy in the dog results in diminished concentration of the serum diastases. The explanation of this fact has not yet been satisfactorily worked out. The hypotheses so far advanced are: (1) the loss of the pancreatic amylopsin, assuming that the serum diastase is pancreatic amylopsin absorbed from the intestine or the pancreas; (2) the absence of the internal secretion of the pancreas, assuming that the serum diastases are produced by all the organs, and that this production is greatly lessened when the internal secretion of the pancreas is wanting. This second hypothesis has recently been advanced by Carlson.

It is also well established that urine contains a diastase or diastases in varying concentrations. The urine diastase is probably taken directly from the blood by the kidneys and therefore identical with the serum diastase. Now, inasmuch as complete pancreatectomy leads to diabetes and polyuria, it is possible that the diminished diastase content of the serum following pancreatectomy is due in part to a more rapid elimination of the diastase by the kidneys. The work here reported was undertaken at Professor Carlson's suggestion with the view of determining the rôle of the kidneys in the regulation of the concentration of the diastases in the serum and the lymphs.

Methods.

One cubic centimetre of serum was added to 10 c.c. of a 1 per cent solution in water of soluble starch previously boiled and cooled to 37° C. Digestion time was determined by the complete disappearance of erythrodextrin, tested at frequent intervals with I in KI.

Diuresis was produced by cutting the renal nerves, and by large quantities of 0.9 per cent salt solution given through stomach tube. Anuresis was effected by ligation of renal arteries.

TABLE I.

DOG. DETAIL OF EXPERIMENT No. 2, SHOWING THE INFLUENCES ON THE DIASTATIC POWER OF THE SERUM OF LIGATION OF THE RENAL ARTERIES.

Date.	Digestion time in hours.	Date.	Digestion time in hours.
June 2	1.10	June 27 5.00 P. M.	1.10
26	1.10	28 8.30 A. M.	1.15
27 8.30 A. M.	1.15	28 10.30 A. M.	1.20
27 9.00 A. M.	Ligation of renal arteries.	28 4.00 P. M.	1.15
27 10.30 A. M.	1.10	29 7.30 A. M.	1.05
27 3.00 P. M.	1.25	29 2.30 P. M.	1.10

To ascertain whether the lowering of the diastatic concentration in marked diuresis is due to a dilution of the blood or to increased rapidity of elimination of the diastase, erythrocyte counts were made of each sample of blood drawn for the diastase tests while the experiment was in progress. The following results were obtained:

I. ANURESIS.

Anuresis was brought about by ligation of renal arteries on both sides in two dogs. One of the experiments is summarized in Table I.

The diastase curve of Dog I is practically identical with the one of Dog II. In both cases the serum was tested for three days following the renal ligation. It seems clear, therefore, that ligation of the renal arteries in the dog has no influence on the diastatic power of the serum. The interpretation of this fact is not clear. Under normal condition serum diastase is constantly eliminated in the urine, and we would expect that when this avenue of elimination is removed the diastase should accumulate in the blood, provided normal rate of production prevailed. It is possible that owing to the toxemia following the ligation there is a diminished production of diastases, or an actual increase in the concentration of the serum diastases is present, but

TABLE II.

Detail of Experiments No. 9 and 10, showing the Influence of Diuresis on the Concentration of the Serum Diastase. The NaCl Solution was given by the Stomach Tube.

	Date.	Blood count, reds.	.9 NaCl thro' tube in c.c.	Vomited in c.c.	Urine recovered in c.c.	Digestion time in hours.
Dog IX	July 25 8.00 A. M.	4,600,000	750	...	...	2.50
	25 8.30 A. M.	4,500,000	...	...	...	....
	25 9.00 A. M.	4,000,000	500	...	50	3.30
	25 9.30 A. M.	....	...	...	50	....
	25 10.00 A. M.	3,600,000	720	...	100	3.40
	25 10.30 A. M.	....	...	150	...	....
	25 11.00 A. M.	3,700,000	800	...	150	3.50
	25 12.00 M.	3,800,000	...	...	550	3.50
	25 1.00 P. M.	4,000,000	...	...	200	3.50
	25 2.30 P. M.	4,000,000	...	...	250	3.50
	25 7.00 P. M.	4,600,000	...	...	...	3.50
	26 8.00 A. M.	4,700,000	...	...	300	3.50
Dog X	7 8.00 A. M.	5,400,000	500	300	...	1.50
	7 9.00 A. M.	5,400,000	500	200	...	1.50
	7 10.00 A. M.	5,000,000	500	150	100	2.20
	7 12.00 M.	4,600,000	500	...	250	2.40
	7 2.00 P. M.	4,600,000	500	...	300	2.50
	7 3.00 P. M.	4,500,000	500	...	500	2.40
	7 7.00 P. M.	4,800,000	...	...	400	2,35
	8 8.30 A. M.	5,500,000	...	...	450	1.55

not capable of demonstration owing to development of depressors or inhibitory substances.

II. DIURESIS

1. **Diuresis produced by section of the renal nerves.** — Only one experiment was carried out by this method. After establishing the normal diastase concentration for a number of days, the renal nerves on both sides were sectioned July 6, and the serum tested every day up till July 13. There was no variation in the diastatic power beyond the daily variations that occur in the normal animal. The polyuria was marked, as long as the dog was under observation.

2. **Diuresis by giving large quantities of physiological salt solution by mouth.** — Nine dogs were used in this series. Blood counts were not made in the first four dogs. The salt solution (0.9 per cent NaCl) was given through a stomach tube. Some of the dogs vomited up portions of the salt solution. On the whole, a 10 kilo dog will retain 2500 c.c. to 5000 c.c. sodium chloride solution given by stomach tube within a period of three hours.

Two typical experiments are given in Table II. In every case the diuresis produced by this method was accompanied by a diminution of the diastatic power of the serum. This is probably not due to an increased diminution of the serum diastase in the urine, for the erythrocyte count shows that there is a sufficient dilution of the blood to account for the change. In fact, the curves of diastase concentration and degree of blood dilution shown by the erythrocyte count run practically parallel. In one case, however, the erythrocyte concentration returned to the normal fifteen hours before the serum had regained its normal diastatic power. This may have been due to too rapid elimination or to diminished production. The dilution of the blood observed in these experiments was obviously due to an intestinal absorption too rapid to be compensated by the kidney secretion.

SUMMARY.

1. In dogs ligation of the renal arteries produces no change in the diastatic powers of the serum.

2. Diuresis caused by section of the renal nerves in the dog does not alter the concentration of the blood diastases.

3. Diuresis due to an excess of salt solution per os is accompanied by a diminution in the diastatic power of the serum, but this appears to be caused by actual dilution of the blood rather than by an increased elimination of the serum diastase by the kidneys.

4. Our results indicate that the polyuria following complete pancreatectomy is not an important factor in the great diminution of the diastase content of the serum observed after this operation.

THE RÔLE OF ANTITHROMBIN AND THROMBOPLASTIN (THROMBOPLASTIC SUBSTANCE) IN THE COAGULATION OF BLOOD.

By W. H. HOWELL.

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IN attempting to explain the coagulation of shed blood we may accept as demonstrated, in spite of the opposition of Nolf, that it is due immediately to a reaction between fibrinogen and the thrombin, whereby fibrin is formed and deposited. In the circulating blood this reaction does not occur because the thrombin though present exists in an inactive form which may be designated as prothrombin or thrombogen, and the secret of coagulation is concealed in the processes which lead to the actual production of thrombin.

Investigations by various observers have demonstrated that two factors are concerned in this formation of active thrombin. In the first place, the presence of calcium — there is practically no difference of opinion upon this point. In the absence of calcium, as in oxalated blood, the blood remains fluid indefinitely. In the second place, an unknown organic substance which may be obtained by extraction from the tissues in general, including the blood corpuscles themselves. The importance of this second factor is perhaps not so generally recognized, but to those who have given much attention to the subject it is as striking as the part taken by calcium. That this influence of tissue extracts is not due to the presence in them of thrombin or of a thrombin-like substance (coagulin) is proved sufficiently by the fact that when added to solutions of fibrinogen, with or without the presence of calcium, they do not produce a clot. The importance of the element furnished by the tissues has been emphasized greatly by a study of the blood of the vertebrates lower than the mammals. Delezenne, Nolf, and others have shown that the blood of birds, reptiles, and fishes when removed carefully from the blood vessels, so as not to come into contact with the tissues, clots very slowly, while contact with the wounded tissues or the addition to the blood of an extract of the tissues causes it to clot in a few minutes. In fact, if in these animals the blood is removed carefully and is then centrifugalized promptly, the clear cell-free plasma clots with extreme slowness or may not clot at all if left to itself, while addition of tissue extracts causes prompt coagulation. The mammalian blood can be brought into an analogous condition by the so-called process of peptonization familiar to all physiologists. According to the success of the "peptonizing" injection the blood exhibits, when drawn at the proper period, either a delayed coagulation time of various degrees or a complete incoagulability. Such peptone bloods or peptone plasmas usually clot quickly when a tissue extract is added, behaving in this respect like the bird's plasma. The efficacy of tissue extracts in inducing or facilitating the clotting of bird's blood, peptone blood, and mammalian blood under normal conditions has found a simple and apparently satisfactory explanation in the theory proposed simultaneously by Morawitz and by Fuld and Spiro. This theory assumes that the organic substance furnished by the tissues, which has been designated by different names, is in fact an activator or kinase that converts the inactive prothrombin to thrombin. For this reaction calcium also is necessary, as is shown by the fact that bird plasma or peptone plasma which is first oxalated to remove the calcium is no longer clotted by the addition of tissue extracts. The importance of the calcium and of the tissue extract is explained on this theory by the assumption that both an inorganic (calcium) and an organic (tissue extract) activator are required to convert prothrombin to thrombin. In shed mammalian blood the organic activator (thrombokinase of Morawitz, cytozym of Fuld and Spiro) is furnished by the quickly disintegrating platelets; in the blood of the lower vertebrates similar corpuscles are lacking, and for such bloods the external tissues must supply the kinase in order to ensure rapid clotting. While this theory is simple, logically complete, and applicable in a very satisfactory way to most of the known phenomena of coagulation, it is evident to any one who gives the subject consideration that another possible explanation may be given to the effect of tissue extracts upon the clotting of bloods of all kinds. Accepting, as must be done, the facts that in the normal blood of all vertebrate animals an antecedent or inactive form of thrombin (prothrombin) is present and also calcium salts, we may suppose that these two fail to react to form thrombin because an inhibiting substance is also present, an antithrombin, which holds the prothrombin and prevents its activation by the calcium. On this view the importance of the tissue extracts lies not in the fact that they contribute a kinase to aid in the activation of the prothrombin, but in the fact that they furnish a substance, which may be designated as a thromboplastic substance or thromboplastin, which neutralizes the antithrombin and thus permits the calcium to activate the prothrombin and start the process of clotting. All the facts that are explained on the assumption that the tissues or the blood corpuscles furnish a kinase are equally well explained on the assumption that they furnish a thromboplastic substance whose function consists in neutralizing an antithrombin, and an effort should be made to test carefully the various significant experimental facts from the standpoint of each of these possible explanations. The author has attempted to devise experiments which might serve to test these mutually exclusive views. The results are presented in the following pages. So far as the author is concerned the work has convinced him that thrombokinase, in the sense used by Morawitz, does not exist, and that the second of the two views stated is much more in accord with the facts that are known in regard to coagulation.

THE PRESENCE OF ANTITHROMBIN IN PEPTONE PLASMA, IN BIRD PLASMA, AND IN NORMAL MAMMALIAN PLASMA.

That a substance which inhibits the action of thrombin on fibrinogen is present in peptone plasma, in bird plasma, and in normal mammalian plasma is easily shown. In fact, it may be said that so far as the peptone plasma is concerned the presence of antithrombin is generally admitted, and the same is true perhaps of bird's plasma (Delezenne, Nolf, Morawitz *et al.*). Morawitz has given experiments to show also that in normal mammalian blood some antithrombin exists. For example, a serum to which some oxalated plasma has been added clots a given specimen of fibrinogen less rapidly than the same serum diluted to an equal amount with a solution of ammonium oxalate. In other words, the oxalated plasma contains something which retards the action of thrombin on fibrinogen. The author has devised a method for obtaining pure thrombin,[1] and by making use of this preparation and of solutions of pure fibrinogen it has been easy to carry out a series of experiments which show in a conclusive way that antithrombin is a constituent of normal mammalian blood, that it is present in larger amounts in the less coagulable bird's blood and in still larger amounts in the incoagulable specimens of peptone blood obtained from dogs. In carrying out these experiments cat's blood, hen's blood, and peptonized dog's blood were used. The cat's blood and hen's blood were each received into a solution of sodium oxalate, 1 per cent, in the proportion of one part of oxalate solution to eight or nine parts of blood, and were then centrifugalized to obtain a clear and cell-free plasma. The peptonized plasma was prepared as described by Cecil in an accompanying paper by injection of the products of an acid digestion of fibrin into the artery of a dog. The blood was withdrawn in ten to fifteen minutes and was centrifugalized to obtain a clear incoagulable plasma. In order to remove from each of these plasmas its own fibrinogen (and prothrombin) it was heated for a few minutes, in a water bath, to 60° C. and then filtered from the resulting precipitate of fibrinogen. These heated plasmas had lost of course all possibility of clotting, but they still contained their antithrombin as shown by such experiments as the following.

Effect of peptone plasma heated to 60° C., and filtered. —

1. Fibrinogen, 1 c.c. Thrombin solution, five drops.	Gives a firm clot in five minutes.
2. Fibrinogen, 1 c.c. Peptone plasma, one drop. Thrombin solution, five drops.	No clot after twenty hours.

Similar preparations in which three and five drops respectively of peptone plasma were added failed to clot.

The peptone plasma was diluted with an equal volume of a solution of sodium chloride 0.9 per cent, and a preparation was made as follows:

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[1] Howell: This journal, 1910, xxvi, p. 453. The principle of the method used to purify the thrombin is to remove other proteins by shaking out with chloroform. The author thought that this method was entirely new, but he has discovered since that Franz used chloroform in a similar way in the preparation of hirudin (Archiv für experimentelle Pathologie und Pharmakologie, 1903, xlix, p. 342).

Fibrinogen, 1 c.c. Peptone plasma, one drop. Thrombin solution, five drops.	The preparation clotted in one hour and thirty-five minutes.

In another series of experiments the oxalated plasma of cat's blood was used instead of solutions of fibrinogen and thrombin to test the inhibiting influence of the peptone plasma. Preparations of the following character were made:

1. Oxalated cat's plasma, ½ c.c. $CaCl_2$ (6 per cent), one drop. Peptone plasma, one drop.	Clotted firmly in five minutes.
2. Oxalated cat's plasma, ½ c.c. $CaCl_2$ (6 per cent), one drop. Peptone plasma, three drops.	Clotted firmly in seven minutes.
3. Oxalated cat's plasma, ½ c.c. $CaCl_2$ (6 per cent), one drop. Peptone plasma, five drops.	Clotted firmly in fifteen minutes.

In a second series the oxalated cat's plasma was diluted with an equal volume of glycerine and water, 1 to 3, this solution being used as a control for parallel experiments with a glycerine solution of thromboplastin to be described below.

4. Oxalated cat's plasma, ½ c.c. Glycerine and water, ½ c.c. $CaCl_2$ (6 per cent), one drop.	Clotted firmly in nine minutes.
5. Oxalated cat's plasma, ½ c.c. Glycerine and water, ½ c.c. Peptone plasma, ten drops. $CaCl_2$ (6 per cent), one drop.	Clotted firmly in eighteen minutes.
6. Oxalated cat's plasma, ½ c.c. Glycerine and water, ½ c.c. Peptone plasma, twenty drops. $CaCl_2$ (6 per cent), one drop.	Not clotted at end of one hour and imperfectly clotted the next morning (twenty hours).

These experiments with similar results reported by other observers show clearly enough that peptone plasma contains something which prevents the reaction between thrombin and fibrinogen. Since this effect is entirely removed by heating the peptone plasma to 80° to 85° C., and since it resembles otherwise the effect of known antithrombin solutions (hirudin), we can explain it most acceptably by assuming that the peptone plasma contains antithrombin and indeed owes its incoagulability to the presence of this substance. The amount of it present in different specimens of peptone plasma shows naturally considerable variation.

Effect of oxalated hen's plasma heated to 60° C., and filtered. — Preparations of the following character were made:

1. Fibrinogen, 1 c.c. Thrombin solution, five drops.	Clotted firmly in eight minutes.
2. Fibrinogen, 1 c.c. Thrombin solution, five drops. Hen's plasma, five drops.	Clotted in thirty-one minutes.
3. Fibrinogen, 1 c.c. Thrombin solution, five drops. Hen's plasma, ten drops.	No clot at end of an hour, and next morning (eighteen hours) only an imperfect membranous clot.
4. Fibrinogen, 1 c.c. Thrombin solution five drops. Hen's plasma, fifteen drops.	No sign of clotting after twenty-four hours.

As proof that the inhibiting action of the hen's plasma was due to an organic substance of the nature of an antithrombin, it was then heated to 80° C., filtered, and the following preparation was made:

5. Fibrinogen, 1 c.c. Thrombin solution, five drops. Hen's plasma (80° C.), ten drops.	Clotted firmly in six minutes.

Effect of oxalated cat's plasma heated to 60° C., and filtered.—This series of preparations was made with the same solutions of fibrinogen and thrombin as those in the series just described with hen's plasma.

1. Fibrinogen, 1 c.c. Thrombin solution, five drops. Cat's plasma, five drops.	Clotted in eighteen minutes.
2. Fibrinogen, 1 c.c. Thrombin solution, five drops. Cat's plasma, ten drops.	Clotted in twenty-five minutes.
3. Fibrinogen, 1 c.c. Thrombin solution, five drops. Cat's plasma, fifteen drops.	An imperfect membranous clot formed after two hours.

The cat's plasma, as in the case of the hen's plasma, was then heated to 80° C., filtered, and tested as follows:

4. Fibrinogen, 1 c.c. Thrombin solution, five drops. Cat's plasma (80 C.), ten drops.	Clotted firmly in eight minutes.

A consideration of these and similar experiments can leave no doubt, it seems to me, that in peptone plasma, bird's plasma, and mammalian plasma there is present a substance of the nature of antithrombin. Comparative experiments with these plasmas in regard to their inhibiting effect upon the same or similar preparations of fibrinogen and thrombin make it clear also that quantitatively the amount of antithrombin is much greater in a good peptone plasma than in bird's plasma, and much greater in the latter than in mammalian (cat's) plasma. The inhibiting influence of the peptone plasma, on the other hand, is much weaker than that of a solution of hirudin made up in the strength of 0.01 gm. to 10 c.c. or 20 c.c. of water or salt solution, that is to say, a solution of 1 to 1000 or 1 to 2000. By making use of a standard solution of hirudin it would probably be quite possible to determine with some exactness the respective amounts of antithrombin present in bird's blood and mammalian blood.

THE EFFECT OF TISSUE EXTRACTS (THROMBOPLASTIN) IN NEUTRALIZING THE ACTION OF ANTITHROMBIN.

As was stated in the introduction to this paper, the well-known effect of tissue extracts in causing or accelerating the coagulation of such plasmas as bird's plasma or peptone plasma may be explained either on the assumption made by Morawitz that such extracts furnish an activating substance for the prothrombin, or on the view that they contain something which neutralizes antithrombin. Experiments were made therefore to determine directly whether or not this last view is tenable.

Preparation of the thromboplastin. — The tissue extracts used in these experiments consisted of a glycerine extract of the spleen or of the thymus such as is described by Cecil in an accompanying paper. The spleen or thymus was first washed with a solution of sodium chloride 0.9 per cent to free it from excess of blood; it was then left for twenty-four hours and ground to a fine pulp with sand. This pulp was spread in a thin layer upon glass and dried thoroughly in a current of warm air (before a register). The dried material was powdered in a mortar, was then rubbed up thoroughly with glycerine, and the glycerine preparation was centrifugalized. Experience showed that prolonged extraction with glycerine does not give so good a preparation as a rapid extraction. The centrifugalized glycerine extract seems to keep indefinitely. When used it is to be diluted with two to three times its volume of water. The dilution produces a turbidity or fine emulsion, and the solution in this condition has a strong thromboplastic action. The glycerine extracts were used as a rule in preference to aqueous or saline extracts of the fresh tissues, because they were more convenient and because in the various experiments the thromboplastic factor could be kept constant by using a single preparation throughout.

To test whether or not the thromboplastin is capable of antagonizing the action of antithrombin two general series of experiments were made. In one series hirudin was used as the antithrombin, and varying amounts of it were tested directly against mixtures of thrombin and fibrinogen or thrombin and oxalated plasma in the presence of a definite amount of thromboplastin. In the other series several different more or less indirect methods were used. The results from this latter series will be described first. In one set of experiments the antithrombin contained in the peptone plasma of dog's blood was used. The clear centrifugalized plasma was first heated for some minutes to 60° C. to get rid of its fibrinogen and prothrombin, and the filtered liquid was used against an oxalated and dialyzed cat's plasma with results of this character.

1. Oxalated plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Six per cent solution $CaCl_2$, one drop	Clotted in nine minutes.
2. Oxalated plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Heated peptone plasma (antithrombin), ten drops. Six per cent solution $CaCl_2$, one drop.	Clotted in eighteen minutes.

3. Oxalated plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Heated peptone plasma, twenty drops. Six per cent solution $CaCl_2$, one drop.	Imperfect clot after one hour.
4. Oxalated plasma, 0.5 c.c. Glycerine extract of thromboplastin (1–3), 0.5 c.c. Heated peptone plasma, ten drops. Six per cent solution $CaCl_2$, one drop.	Clotted in five minutes.
5. Oxalated plasma, 0.5 c.c. Glycerine extract of thromboplastin (1–3), 0.5 c.c. Heated peptone plasma, twenty drops. Six per cent solution $CaCl_2$, one drop.	Clotted in eight minutes.

In this experiment the retarding effect of the heated peptone (2 and 3) plasma due to its antithrombin was neutralized by the addition of the glycerine extract of thromboplastin (4 and 5). The acceleration in the time of clotting caused by the thromboplastin cannot be explained by assuming that it served to activate the prothrombin in the oxalated plasma, since the control experiment (1) with calcium chloride showed that this substance alone was sufficient for this purpose.

A second set of experiments in which hirudin was used for the antithrombin gave similar results. The hirudin was the commercial preparation and was used in solutions which contained 0.01 gm. to 10 c.c. of normal saline.

1. Oxalated plasma, 0.5 c.c. Solution NaCl, 0.9 per cent, 0.5 c.c. Six per cent solution $CaCl_2$, 1 drop.	Clotted in ten minutes.
2. Oxalated plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Hirudin, one drop. Six per cent solution $CaCl_2$, one drop.	Clot formed after one hour.
3. Oxalated plasma, 0.5 c.c. Glycerine extract of thromboplastin (1–3), 0.5 c.c. Hirudin, one drop. Six per cent solution $CaCl_2$, one drop.	Clotted in ten minutes.

Here, again, the acceleration in the clotting caused by the thromboplastin (3 compared with 2) cannot be due to its action in activating the prothrombin in the oxalated plasma, since the control (1) indicates that the calcium alone sufficed for this activation.

In a third set of experiments a preparation containing both prothrombin and antithrombin was made by heating a specimen of clear peptone plasma to 55° C. for a brief period, just sufficient to precipitate the fibrinogen; this plasma was then filtered and the following experiments were made with it and a solution of pure fibrinogen, prepared from cat's plasma. For convenience the heated peptone plasma containing prothrombin may be designated P.

1. Solution of fibrinogen, 0.5 c.c. Solution P., six drops. Six per cent solution $CaCl_2$, one drop.	Clotted in fifty-five minutes.
2. Solution of fibrinogen, 0.5 c.c. Solution P., six drops.	No clot formed.
3. Solution of fibrinogen, 0.5 c.c. Solution P., six drops. Glycerine ex't of thromboplastin, 0.5 c.c. Six per cent solution $CaCl_2$, one drop.	Clotted in nine minutes.

In these experiments the great acceleration in the time of clotting caused by the solution of thromboplastin may have been due possibly either to its hastening the activation of the prothrombin, according to the theory of Morawitz, or it may have been due to its neutralizing the influence of the anti-thrombin which was contained in solution P. That this latter explanation is the correct one is made most probable by the following additional experiments in which a solution of pure thrombin was used.

1. Solution of fibrinogen, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Solution of thrombin, six drops.	Clotted in nine to ten minutes.
2. Solution of fibrinogen, 0.5 c.c. Glycerine extract of thromboplastin, (1–3), 0.5 c.c. Solution P., five drops. Six per cent solution $CaCl_2$, one drop.	Clotted in nine to ten minutes.

3. Solution of fibrinogen, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Solution P., five drops. Solution of thrombin, six drops.	Clotting after one hour, clot incomplete.

In this series Experiment 3 contained the same quantity of thrombin as No. 1 and should therefore have clotted in the same time. The fact that it did not is explained by the presence of antithrombin in the solution P (prothrombin).

In Experiment 2 clotting occurred promptly, because the thromboplastin added neutralized the antithrombin in P.

If one adopts the other view, namely, that the thromboplastin solution simply accelerated the activation of the prothrombin, then the fact that in No. 3 coagulation was so much delayed is not explained, since in this experiment as much thrombin was added as in No. 1. A further demonstration that the thromboplastin acted by neutralizing the antithrombin present in P was given by an experiment in which P was heated sufficiently high to destroy its contained antithrombin (85°).

4. Solution of fibrinogen, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Sol'n P. after heating (85°), five drops. Solution of thrombin, six drops.	Clotted in fourteen minutes.

While these experiments make it highly probable that tissue extracts influence coagulation by antagonizing or neutralizing the antithrombin constantly present in blood, it is evident that more decisive proof for this view may be obtained from experiments in which definite amounts of thrombin and antithrombin are balanced against each other in a calcium-free solution of fibrinogen or in an oxalated plasma. Under such conditions the influence of the tissue extracts should be manifest if, as is assumed, their contained thromboplastin neutralizes in some way the retarding effect exercised by antithrombin upon the union of thrombin and fibrinogen. Under such conditions it will be noted that the tissue extracts cannot act as a kinase in the sense used by Morawitz, since by his hypothesis the thrombokinase is ineffective in the absence of calcium, and in the solutions employed no calcium was added except what may have been present in the glycerine extract of the tissue. Control experiments showed that this extract when used alone was totally unable to cause the clotting of a dialyzed oxalated plasma.

The idea of a neutralization relationship between antithrombin and tissue extracts (thromboplastin) has been tested by other observers. Fuld and Spiro[2] and Morawitz[3] state specifically that no such relationship exists, but in view of the results presented below it seems probable that in their experiments the antithrombin was used in too great a concentration. So also Schittenhelm and Bodong[4] state that tissue extracts do not prevent the inhibiting action of hirudin added to solutions of fibrinogen and serum, but if a centrifugalized hirudin plasma is used then tissue extracts do neutralize the hirudin and cause clotting. The difference in results by these two methods may have been due to the fact that in the centrifugalized hirudin plasma the hirudin was present in smaller concentration, or, as is indicated by my experiments, it may be due to the fact that the relationship between fibrinogen, thrombin, and antithrombin is not quantitatively the same in the case of solutions of fibrinogen, isolated by several precipitations, and solutions of fibrinogen as they exist normally in the blood. That is to say, the restraining effect which antithrombin exercises upon the reaction between fibrinogen and thrombin is more marked when purified solutions of fibrinogen are used than in the case of a normal plasma.

In the first experiments made by the author to test the neutralization relationship between antithrombin and thromboplastin apparently negative results were obtained owing to the fact that the solutions of antithrombin used (hirudin) were too concentrated. When this mistake was rectified by a proper dilution of the hirudin solutions, the fact that such a neutralization relationship exists was clearly demonstrated, as appears from the following series of experiments:

Experiment, April 4. — Materials used: Solution of fibrinogen, solution of hirudin (1–40,000), solution of pure thrombin, glycerine extract of thymus (thromboplastin).

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[2] Fuld and Spiro: Hofmeister's Beiträge, 1904, v, p. 171.

[3] Morawitz: Archiv für klinische Medizin, lxxix, p. 432, and Ergebnisse der Physiologie, 1905, iv, p. 307.

[4] Schittenhelm and Bodong: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liv, p. 217.

1. Fibrinogen, 0.5 c.c.
   Hirudin, one drop.
   Thromboplastin, 0.5 c.c.
   Thrombin, five drops.
   Clotted in less than an hour.
2. Same mixture except hirudin, two drops, clot in three hours.
3. Same mixture except hirudin, three drops, no clot.
4. Same mixture except hirudin, four drops.
5. Same mixture except hirudin, five drops.
   No clot.

I. Fibrinogen, 0.5 c.c.
   Hirudin, one drop.
   Glycerine and water (1–3), 0.5 c.c.
   Thrombin, five drops.
   Clot in two hours, fifteen min.

II. Same mixture except hirudin, two drops, feeble clot in six hours, thirty minutes.

III. Same mixture except hirudin, three drops.
IV. Same mixture except hirudin, four drops.
V. Same mixture except hirudin, five drops.
No clot.

Comparison of Nos. 1 and 2 with I and II shows that in the presence of the thromboplastin the process of clotting was greatly accelerated. A similar result was obtained with the same mixtures in which the hirudin was twice as concentrated (1–20,000).

Experiment, May 9. — Solutions used: Oxalated plasma of cat's blood, centrifugalized and dialyzed, solution of hirudin, 1–20,000, solution of pure thrombin, saline extract of (0.7 per cent NaCl) washed terrapin's muscle (thromboplastin).

1. Plasma, 0.5 c.c.
   Hirudin, two drops.
   Thromboplastin, 0.5 c.c.
   Thrombin, five drops.
   Clot in one-half hour.
2. Same mixture except hirudin, four drops, clot in two hours, fifteen minutes.
3. Same mixture except hirudin, six drops, clot next morning (twenty hours).
4. Same mixture except hirudin, eight drops, clot next morning (twenty hours).

I. Plasma, 0.5 c.c.
   Hirudin, two drops.
   Sodium chloride (0.7 per cent), 0.5 c.c.
   Thrombin, five drops.
   Imperfect clot after two to three hours. Not increased after twenty-four hours.

II. Same mixture except hirudin six drops, no clot after twenty-four hours. This experiment furnishes very striking evidence of the effect of a tissue extract in neutralizing the influence of the hirudin upon the process of clotting. The only objection that might be raised to its conclusiveness is that the saline extract of terrapin's muscle may have contained some calcium salts, and these together with the tissue extract (thrombokinase) may have activated the prothrombin in the plasma used. The very small amount of calcium in muscle makes this suggestion improbable, and it is ruled out entirely by the fact that specimens of the plasma plus the saline extract of muscle showed no signs of clotting at all.

Experiment, May 10. — The same solutions were taken as in the previous experiment except that for the thromboplastin a glycerine extract of thymus was used.

1. Plasma, 0.5 c.c.
Thromboplastin, 0.5 c.c.
Thrombin, five drops.
Hirudin, four drops.
} Clotted within thirty minutes.

2. Same mixture except hirudin, six drops, clotted in one hour, twenty-five minutes.

3. Same mixture except hirudin, eight drops, not clotted after one hour, thirty minutes, but clotted next morning (sixteen hours).

I. Plasma, 0.5 c.c.
Glycerine and water (1–3), 0.5 c.c.
Thrombin, five drops.
Hirudin, four drops.
} Not clotted after one hour, thirty minutes. Found clotted next morning (sixteen hours).

II. Same mixture except hirudin, six drops, not clotted after one hour, thirty minutes, but found clotted next morning (sixteen hours).

III. Same mixture, except hirudin, eight drops, not clotted after twenty hours.

Experiment, May 17. — Solutions used: Cat's oxalated plasma, centrifugalized and dialyzed, solution of hirudin, 1–20,000, solution of pure thrombin, glycerine extract of thymus (thromboplastin).

1. Plasma, 0.5 c.c.
Glycerine and water (1–3), 0.5 c.c.
Thrombin, five drops.
Hirudin, two drops.
} Clotted in twenty-six minutes.

I. Plasma, 0.5 c.c.
Thromboplastin, 0.5 c.c.
Thrombin, five drops.
Hirudin, two drops.
} Clotted in fifteen minutes.

2.	Plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Thrombin, five drops. Hirudin, three drops.	Clotted in thirty-six minutes.
II.	Plasma, 0. 5 c.c. Thromboplastin, 0.5 c.c. Thrombin, five drops. Hirudin, three drops.	Clotted in twenty-two minutes.
3.	Plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Thrombin, five drops. Hirudin, four drops.	Clotted in fifty minutes.
III.	Plasma, 0.5 c.c. Thromboplastin, 0.5 c.c. Thrombin, five drops. Hirudin, four drops.	Clotted in thirty minutes.
4.	Plasma, 0.5 c.c. Glycerine and water (1–3), 0.5 c.c. Thrombin, five drops. Hirudin, eight drops.	Not clotted in five hours. Clotted next morning, twenty-two hours.
IV.	Plasma, 0.5 c.c. Thromboplastin, 0.5 c.c. Thrombin, five drops. Hirudin, eight drops.	Clotted in three hours, thirty minutes.

This experiment exhibits very completely the influence of the thromboplastin in neutralizing the effect of antithrombin; it also shows a fact which was made evident throughout the whole series of experiments, namely, that this neutralizing action of the thromboplastin is most striking when the proportion of antithrombin used is such as to greatly slow the action of thrombin upon the fibrinogen without entirely preventing this action. On the other hand, when sufficient antithrombin was used to entirely prevent coagulation for twenty-four hours or more, it was difficult to so graduate this excess as to overcome it with a solution of thromboplastin, remembering that this latter solution was added to the extent of 0.5 c.c. It is probable that this difficulty may have been due to the fact that the thromboplastin solutions used were in reality quite dilute compared with those of the hirudin, so that in increasing the amount of the latter added to the mixtures the proportion of hirudin (antithrombin) was augmented relatively too fast.

Experiment, May 30. — Solutions used: Cat's oxalated plasma, centrifugalized and dialyzed — (forty-eight hours old) — solution of hirudin, 1–20,000 — solution of pure thrombin, extract of pigeon's testis made in solution of NaCl, 0.9 per cent with sodium oxalate added to 0.1 per cent.

1.	Plasma, 0.5 c.c. Hirudin, one drop. Testis extract, 0.5 c.c. Thrombin, five drops.	Clot in ten minutes.
I.	Plasma, 0.5 c.c. Hirudin, one drop. Sodium chloride and sodium oxalate, 0.5 c.c. Thrombin, five drops.	Clot in ten minutes.

2. Same mixture as (1), but hirudin, two drops, clot in thirty-five minutes.

II. Same mixture as I, but hirudin, two drops, small membranous clot in three hours. Firm clot at twenty hours.

3. Same mixture as (1), but hirudin, four drops, not clotted in twenty-four hours.

III. Same mixture as I, but hirudin, four drops, not clotted in twenty-four hours.

This experiment is of interest, since the tissue extract was made with an oxalated solution of sodium chloride, hence entirely free from available calcium. The neutralizing effect of this extract on the hirudin is, however, evident in 2 as compared with II.

A similar set of experiments was made with these same solutions except that the thromboplastin was supplied by a glycerine extract of thymus. These experiments are to be compared with I, II and III.

A-I.	Plasma, 0.5 c.c. Hirudin, one drop. Thromboplastin, 0.5 c.c. Thrombin, five drops.	Clot in ten minutes.
A-II.	Plasma, 0.5 c.c. Hirudin, two drops. Thromboplastin, 0.5 c.c. Thrombin, five drops.	Clot in forty minutes.
A-III.	Same mixture, but hirudin, three drops.	Clot in one hour, twenty minutes.
A-IV.	Same mixture, but hirudin, four drops.	Clot in three hours.

Experiment, May 31. — Solutions used: Cat's oxalated plasma (seventy-two hours old), solution of hirudin, 1–20,000, solution of pure thrombin, solution of thromboplastin, glycerine extract of thymus.

1. Plasma, 0.5 c.c.
Hirudin, two drops.
Glycerine and water (1–3), 0.5 c.c.
Thrombin, five drops.
Clotted within fifteen minutes.

I. Plasma, 0.5 c.c.
Hirudin, two drops.
Thromboplastin, 0.5 c.c.
Thrombin, five drops.
Clotted within fifteen minutes.

2. Same mixture as (1), but hirudin, three drops, clotted in thirty-five minutes.

II. Same mixture as I, but hirudin, three drops, clotted within fifteen minutes.

3. Same mixture as (1), but hirudin, four drops, clotted in thirty-five minutes.

III. Same mixture as I, but hirudin, four drops, clotted in forty minutes.

4. Same mixture as (1), but hirudin, five drops, not clotted after six hours, but found clotted next morning.

IV. Same mixture as I, but hirudin, five drops, clotted in one hour, fifty minutes.

5. Same mixture as (1), but hirudin, six drops, not clotted after twenty-four hours.

V. Same mixture as I, but hirudin, six drops, not clotted in five hours, but found clotted next morning.

In this series the accelerating effect of the thromboplastin is shown, except in Nos. 3 and III, in which the control clotted somewhat more promptly. Later an additional series of tests were made as follows:

1. Plasma, 0.5 c.c.
Hirudin, two drops.
Glycerine and water (1–3), 0.5 c.c.
Thrombin, five drops.
Clotted in twenty-five minutes.

I. Plasma, 0.5 c.c.
Hirudin, two drops.
Thromboplastin, 0.5 c.c.
Thrombin, five drops.
Clotted in fifteen minutes.

2. Same mixture as (1), but hirudin, four drops, clotted in two hours, twenty minutes.

II. Same mixture as I, but hirudin, four drops, clotted in fifty minutes.

3. Same mixture as (1), but hirudin, six drops, not clotted in twenty-four hours.

III. Same mixture as I, but hirudin, six drops, not clotted in twenty-four hours.

Taking .these series of experiments as a whole, they seem to the author to show conclusively that the material in tissue extracts, thromboplastin, accelerates the act of clotting in mixtures containing antithrombin, and no explanation of this accelerating action is possible other than the view that the thromboplastin neutralizes in some way the inhibiting influence exerted by the antithrombin. Morawitz's hypothesis that the thromboplastin functions as a kinase which in combination with calcium activates some of the prothrombin present in the plasma used is not permissible for several reasons. In the first place the effect is shown also when calcium-free solutions of fibrinogen are used in conjunction with active thrombin (experiment, April 4). In the second place no calcium was present in these solutions to act with the kinase, except possibly a minute amount which may have been contained in the 0.5 c.c. of thromboplastin solution used. That the calcium, if any, contained in these solutions was not sufficient for such a purpose is indicated by the fact that the thromboplastin solutions added to the oxalated plasma in equal amounts caused no clotting whatever, and moreover in Experiment, May 30, part 1, the tissue extract was itself oxalated to remove any calcium that may have been present.

In what way the thromboplastin neutralizes the action of the antithrombin is not immediately apparent from these experiments. The simplest hypothesis is the assumption that the thromboplastin combines with the antithrombin and thus breaks up the combination between the latter and the thrombin. Observations made upon so-called peptone plasma (Fano) in which the incoagulability is due to the presence of antithrombin show that this plasma may be made to clot by simple dilution with water, by the addition of acids, by the action of carbon dioxide, etc. So also the author has shown that incoagulable terrapin's plasma, obtained by centrifugalizing the blood which has been caught without contact with the tissues, may be made to clot on dilution with water, although dilution with normal saline (NaCl 0.9 per cent) is without effect. It would appear from these observations that in such bloods the combination between the antithrombin and the thrombin, or rather the combination between the antithrombin and the prothrombin, is one that can be dissociated with some ease, and quite possibly therefore the thromboplastin effects this dissociation, when the concentration of the antithrombin is not too great, and thus liberates the thrombin or the prothrombin as the case may be. In coagulable liquids containing fibrinogen, thrombin, antithrombin, and thromboplastin, we have the conditions for a reaction which may tend in one direction or the other according to the concentration of the reacting constituents. The thrombin may combine with the fibrinogen or with the antithrombin, the latter may combine with the thrombin or with the thromboplastin, while the fibrinogen combines only with the thrombin and the thromboplastin combines only with the antithrombin.

While the fibrinogen can combine with thrombin but not with prothrombin, there seems to be no doubt that the antithrombin combines with the prothrombin as well as the thrombin, and indeed in peptonized plasma, in bird's blood, and even in mammalian blood the antithrombin which is undoubtedly present is probably combined with the prothrombin. At various times in these experiments the author has obtained results which seemed to indicate that the combination between antithrombin and prothrombin is more easily dissociated, by thromboplastin for example, than the combination between antithrombin and thrombin.

Application of the Foregoing Results to the Normal Coagulation of Blood.

If, as the experiments given seem to prove, a relation of neutralization does exist between thromboplastin and antithrombin, and if moreover, as also seems to be clearly demonstrated, antithrombin is a normal constituent of the circulating blood in mammals as well as in the lower vertebrates, then it is evident that the alternative theory of the normal process of clotting, proposed at the beginning of this paper, is worthy of serious consideration. The hypothesis that the tissue elements or the white corpuscles of the blood furnish an enzyme or kinase which can activate prothrombin only in the presence of, or with the assistance of, calcium does not rest upon direct evidence. To obtain direct evidence for this hypothesis, or indeed to obtain conclusive evidence against it, it will be necessary to secure pure prothrombin and ascertain whether or not it is activated by calcium salts alone. But the alternative hypothesis that the tissue extracts (including the material obtainable from disintegrating leucocytes or blood plates) promote or initiate the clotting of blood not by activating the prothrombin but by neutralizing the antithrombin has the direct experimental basis furnished in this paper.

Looking at the matter from this standpoint, the process of normal coagulation may be pictured as follows. In the circulating blood we find as constant constituents, fibrinogen, prothrombin, calcium salts, and antithrombin. The last-named substance holds the prothrombin in combination and thus prevents its conversion or activation to thrombin. When the blood is shed, the disintegration of the corpuscles (platelets) furnishes material (thromboplastin) which combines with the antithrombin and liberates the prothrombin; the latter is then activated by the calcium and acts upon the fibrinogen. According to this view the actual process of coagulation involves only three factors, fibrinogen, prothrombin, and calcium. These three factors exist normally in the circulating blood, but are prevented from reacting by the presence of antithrombin. In the bloods of the vertebrates lower than the mammals the blood, when not brought into contact with the external tissues, clots very slowly, and when caught in clean vessels and promptly centrifugalized the resulting plasma, free from corpuscular elements, may be entirely incoagulable. In these animals, therefore, effective clotting, such as is necessary to protect them from fatal hemorrhage, depends upon the cooperation of the wounded tissues which furnish the thromboplastin required to neutralize the antithrombin, this latter substance being present in these bloods in greater concentration than in the mammalian blood. From this point of view the mammalian blood differs from the bloods of the other vertebrates in that it contains within itself an available source of thromboplastin, most probably in the existence of the easily disintegrating platelets which are not found in the blood of the other vertebrates.

It will be noted that according to this hypothesis the circulating blood contains normally the three necessary factors of coagulation and its fluidity is maintained by the presence of the antithrombin. Various observers have at times been forced to the conclusion that the circulating blood contains within itself all the factors necessary for coagulation, and the present author has called attention to an observation or experiment which seems to demonstrate this fact, as follows. If the slowly coagulating blood of the terrapin is received, without coming into contact with the tissues, into vaselined tubes, is kept at a low temperature, and while cold is thoroughly centrifugalized, a clear plasma may be obtained which will remain unclotted for days or perhaps indefinitely. Nevertheless this plasma clots very promptly upon simple dilution with water. The conditions of the experiment make it probable that none of the corpuscular elements in the blood have undergone disintegration during the process of separating off the plasma, and since the plasma coagulates upon simple dilution with water it follows that it must contain all the factors of coagulation, only under normal circumstances they are prevented from reacting. A fact of this kind is difficult to explain upon the theory of Morawitz and similar theories which assume that in the circulating blood some one factor, *e. g.*, thrombokinase, is lacking. It is explicable on the hypothesis here proposed if we assume, as is justifiable from other grounds, that the combination between antithrombin and prothrombin upon which the fluidity of the blood depends, is dissociated or weakened by dilution with water. The same suggestion may be used perhaps to explain the well-known observation of Bordet and Gengou.[5] According to these observers mammalian blood received into paraffined vessels and thoroughly centrifugalized yields a cell-free plasma which remains unclotted as long as it is retained in paraffined vessels, but clots when transferred to a glass vessel or other receptacle which the plasma can wet. This observation is in line with the numerous experiments of Nolf[6] which emphasize the importance in coagulation of the presence of finely divided particles in the coagulable liquid. Such particles, to use Nolf's phrase, act as centres of coagulation and inaugurate the process of clotting. This fact is indeed beautifully illustrated, as Rettger[7] has shown, in

[5] **Bordet and Gengou**: Annales de l'Institut Pasteur, 1903, xvii, p. 822.

[6] **Nolf**: Archives internationales de physiologie, 1908, vi, p. 115.

[7] **Rettger**: This journal, 1909, xxiv, p. 429.

the case of the bird's blood when withdrawn from the vessels so as not to come into contact with the tissues. This blood or the plasma obtained from it may be made to clot promptly by the addition of mechanical particles such as dust, bits of feathers, etc. All of these observations upon the accelerating effect of surface contact may be explained on the hypothesis that the combination of antithrombin and prothrombin is thereby dissociated. It is to be noted, however, that this physical factor or the effect of dilution is effective only in those plasmas in which the amount of antithrombin is small. A good peptonized plasma or hirudinized plasma will not clot upon the addition of material particles, and with difficulty, if at all, upon dilution with water.

The view here proposed of the cause of the coagulation of shed blood assumes that the circulating blood is at all times ready to clot as soon as the antithrombin is neutralized, and it therefore exalts the importance of this latter substance. As Morawitz has stated, the variations in coagulation time in different individuals and under different conditions, normal and pathological, may well depend upon variations in the supply of antithrombin. Under normal conditions there must be sufficient antithrombin present to hold the prothrombin, and yet a large excess is promptly removed by excretion or otherwise, as is shown by the fact that in peptonized dogs the diminished coagulability of the blood, caused by the over-production of antithrombin, disappears after an hour or so. It would appear therefore that in this relationship between antagonistic substances, as in other similar relations in the body, automatic regulating mechanisms exist that control the balance between the prothrombin and antithrombin. The fact that excess of thrombin (see accompanying paper by Davis) or of prothrombin yielding material (leucocytes) when injected into the circulation causes an almost immediate output of antithrombin into the blood, suggests the possibility that a part at least of this automatic regulation may be due to the fact that the prothrombin or thrombin itself constitutes a hormone which excites the secretion of antithrombin.

SUMMARY.

1. By the use of solutions of pure fibrinogen and thrombin it is shown that an antithrombin is present in normal mammalian plasma, as well as in bird's plasma and in the so-called peptone plasma.

2. Tissue extracts contain a substance (thromboplastin) which neutralizes the effect exerted by antithrombin upon the reaction between fibrinogen and thrombin.

3. On the basis of the foregoing results the following hypothesis is suggested to explain the normal coagulation of blood. Circulating blood contains normally all the necessary fibrin factors, namely, fibrinogen, prothrombin, and calcium. These substances are prevented from reacting, and the normal fluidity of the blood is maintained, by the fact that antithrombin is also present, and this substance prevents the calcium from activating the prothrombin to thrombin. In shed blood the restraining effect of the antithrombin is neutralized by the action of a substance (thromboplastin) furnished by the tissue elements. In the mammalia the thromboplastin is derived, in the first place, from the elements of the blood itself (blood platelets). In the lower vertebrates the supply of this material, in normal clotting, comes from the external tissues.

NOTES ON THE CREATININE EXCRETION OF THE PIG.

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WITH the discovery by Folin[1] that the amount of creatinine excreted by an individual is nearly constant, together with his contribution of a quantitative method for its determination, which in accuracy and ease of operation leaves little to be desired, we are in possession of a valuable aid to the study of certain phases of protein metabolism hitherto not possible. The work of Folin leaves little doubt that there are normally two kinds of protein metabolism going on simultaneously in the animal body: one, the essential or endogenous, is practically constant and represents the processes of cellular activity; the other, the exogenous, which is very variable and represents the prompt conversion of the nitrogen of the food protein into the end products of metabolism, principally urea. The magnitude of this type depends upon the protein intake and at moderately high levels is proportional to it. The endogenous type results in the formation of a distinct group of end products, the only one of which we have any definite knowledge being creatinine.

Folin, working with men, did not attempt to entirely eliminate the exogenous type of metabolism by long-continued feeding of a ration very low in nitrogen. After ten days on diets containing less than a gram of nitrogen a day, the men in his experiments still eliminated more than 60 per cent of the total nitrogen of the urine as urea. Since urea is the nitrogenous constituent suffering the most marked diminution in quantity as the exogenous metabolism is diminished, it did not appear from his experiments how nearly the latter type had been eliminated. In order to arrive at a condition in which the whole of the protein metabolism is of the endogenous type, provided this is possible, it would be necessary to keep an animal during a long period on a diet free from nitrogen, but supplying all the other essential elements and organic complexes necessary to normal metabolism. Doubtless also these things should be supplied in generous but not excessive amounts, and the animal should be kept under as nearly normal conditions as possible. These conditions are difficult to realize with any animals ordinarily employed in metabolism studies. A considerable experience with the pig as a subject in our work with the mineral elements led me to the belief that this animal would be unusually valuable for studies in protein metabolism. This belief was strengthened by the observation that, when kept in a cage, a vigorous pig will take a sufficient quantity of a solution of starch containing the necessary salts to meet all its energy requirements, day after day, with no evidence of anorexia and with no appreciable loss in weight. In an experience with more than a dozen animals, extending over a period of two and a half years, I have found only two animals which proved unsatisfactory in this respect.

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[1] Folin: This journal, 1905, xiii, p. 84.

Preliminary to a series of experiments in which it was desired to feed quantities of nitrogen equivalent to the endogenous nitrogen metabolism of the animal, an examination was made of the possibility of using the creatinine excretion as an index to the amount of nitrogen derived daily from this source. It was believed that if an animal was given a liberal energy supply in the form of starch, and a salt mixture containing all the essential radicals, and water, it would ultimately reduce its exogenous protein metabolism to nearly, if not quite, the vanishing point. In this condition the ratio of creatinine N to total N in the urine should become constant. If this constant ratio should be confirmed for a sufficient number of animals, it would be a very valuable one, since to determine the nitrogen from endogenous metabolism it would only be necessary to determine the creatinine in the urine for a number of days, to arrive at an average value, and multiply the nitrogen appearing in this form by the factor derived from the constant ratio.

The results of experiments with seven animals are recorded in Table I. Since only the last portions of the records are of interest, only the body weight, length of the period on a nitrogen-free diet, average nitrogen content of the urine during the last five days, average content of creatinine nitrogen and its ratio to the total nitrogen are given. It will be seen that the ratio of creatinine nitrogen to total nitrogen finally established is between 17.8 and 19.2, to 100. The average of, all the experiments except the last is 18.5. In the case of the pig showing a ratio of 22 to 100 the pig had been killed before the results were calculated. Only the colorimeter readings had been recorded at the time of the experiments and the calculations were made at a later date owing to the pressure of other duties. It was, therefore, not possible to try this pig again. However, the ratios from the other animals fall within so narrow a range that their average 18.5 may be safely accepted as a close approximation to the final ratio on the level where the metabolism remains constant.

TABLE I.

Showing the Ratio between the Nitrogen as Creatinine and the Total Nitrogen in the Urines of Pigs after long-continued Nitrogen Free Diet.

No. of pig.	Initial weight.	Days on N free diet.	Av. N content of urine last five days.	Av. creatinine N in urine last five days.	Per cent of total N as creatinine.
	lb.				
16	24.0	27	0.54	.104	19.25
21	85.0	24	1.83	.336	18.36
9	150.5	24	2.65	.471	17.77
8	43.5	36	1.09	.206	18.50
5	37.0	21	0.90	.160	17.77
10	82.0	24	1.64	.314	19.16
7	165.0	23	2.61	.574	22.00

The animals lost but little weight in any case, and in a number the weight remained constant throughout the experiment. The time required to attain this ratio depends on the previous state of nutrition and on the volume of urine eliminated. A high output of urine tends to a more rapid attainment of the minimum level of nitrogen excretion. I have not observed the attainment of the constant ratio in any case in less than about sixteen days.

The fact that a nearly constant ratio of creatinine nitrogen is established under the conditions of these experiments does not, of course, necessarily mean that all of the nitrogen in the urine is derived from endogenous sources. An examination of several of these urines showed that about 60 per cent of the total nitrogen was still present in the form of urea. Creatine was absent entirely.

Urea may also be a product of endogenous metabolic processes, or may result from the further decomposition of unknown bodies so derived. However this may be, the nitrogen eliminated by the animal when this nearly constant ratio is reached, represents the absolute minimum level of protein metabolism of which the animal is capable, and if nitrogen equilibrium is to be maintained, at least this amount of nitrogen must be supplied in the food and in a utilizable form. We are justified in assuming this nitrogen to be derived wholly from endogenous sources, until further information is gained.

If we find the average amount of nitrogen appearing in the urine during four or five days when the pig is taking no food from animal sources (that is, a creatine and creatinine free diet) and multiply this by 5.5, the product will closely approximate the amount of nitrogen which the animal would eliminate daily in the urine if kept for a long period on a nitrogen-free diet. It is safe to accept this as the maintenance level.

Whether this factor will also apply to all species of mammals as well as it does to the pig cannot be told at the present time, since no other species has as yet been investigated. Some investigators have not found the creatinine excretion as constant in the carnivora (dog) as has been found by Folin with men and by myself with the pig. Folin[2] has suggested that the endogenous metabolism of the carnivora may not be as constant as in other types of animals.

Another way in which the creatinine excretion will without doubt be of very great value in nutrition studies is in serving as a basis for the calculation of rations in animals employed in exact nutrition studies. In the past it has been the custom of investigators to base the calculation of rations on the body weight of the animal. This method has always been known to have little merit, because of the great variation in the fat content of the body. In creatinine we have an end product of cellular activity which is eliminated with surprising regularity, and while the amount eliminated is only roughly proportional to the body weight it seems to be, as Folin pointed out, closely proportional to the amount of metabolizing tissue in the body. If, therefore, it is desired to feed two animals comparable quantities of a substance, as protein, it would undoubtedly be much more accurate to feed them the same multiple of their respective creatinine nitrogen excretion. This method is being used in this laboratory in work now in progress on the efficiency with which the growing pig utilizes certain forms of nitrogen for growth.

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[2] FOLIN: This journal, 1905, xiii, p. 129.

TABLE II.

SHOWING THE RISE IN THE CREATININE OUTPUT ATTENDING THE RETENTION OF A DEFINITE AMOUNT OF NITROGEN IN THE GROWING PIG.

N retained.	Rise in creatinine N.	Grams N retained corresponding to a rise of 1 mgm. of creatinine N output.
gm.	gm.	
175	.073	2.39
85	.039	2.18[1]
329	.134	2.46

[1] This pig was sick at the close of this experiment and failed one day to void any urine, during the last six days from which the final creatinine output was estimated. This disturbance in the record renders the figure of uncertain value.

It seemed to me worth while to study the relationship between the rise of creatinine elimination and the retention of nitrogen during growth in the pig. Data on this point have been collected with only three pigs, and the results are presented here (Table II) without any expression as to finality, since they may need revision in the light of further study. Further work is being done on this subject.

THE NATURE OF THE REPAIR PROCESSES IN PROTEIN METABOLISM.

By E. V. McCOLLUM.

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AS a result of the great advances in our knowledge of the chemistry of the proteins in recent years, numerous problems relating to protein metabolism in the animal have arisen. It is now known that proteins from various sources differ widely from one another in fundamental characters, and that all are not equivalent as nutrients for animals. Casein and vitellin have each been shown to be capable of maintaining an animal in nitrogen equilibrium, and recently Osborne, Mendel, and Ferry[1] have added glutenine from wheat to the list of proteins which are individually capable of meeting all the needs of an animal, at least so far as maintenance is concerned, although up to the present time no very decided positive nitrogen balances have been reported in experiments where the animals were limited to a single protein as a source of nitrogen. Zein, gelatin, and others have been found not to possess this power.

The prevailing views of the mechanism of protein metabolism have been supported and developed principally by Abderhalden. The amino acids, which all proteins yield on hydrolysis, are regarded as the "building stones" with which the animal deals in constructing its specific body proteins. It is assumed that the animal has no synthetic power which enables it to produce from other complexes the amino acids needed, with the single exception of glycocoll. Henriques[2] has brought forward experimental evidence which conflicts with this view, in that he kept rats in nitrogen equilibrium with a ration containing no protein other than gliadin, from which lysin is absent.

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[1] OSBORNE and MENDEL and FERRY: Bulletin 156, of the Carnegie Institution of Washington, D. C., 1911.

[2] HENRIQUES: Zeitschrift für physiologische Chemie, 1909, lx, p. 105.

Abderhalden[3] has disputed the possibility of accomplishing this with strictly pure gliadin.

From this view of the nature of protein metabolism it follows that the greater the similarity of the molecule of food protein to that of the specific body proteins, the greater will be the food value to the animal. It also follows that any one of the essential cleavage products which is present in smallest amount in the food protein determines the value of the entire molecule to the animal.

The most elaborate effort to test the validity of this hypothesis is the work of Michaud,[4] who says: "Man nur dann dem Eiweissminimum am nächsten kommt, wenn man das körpereigene Eiweiss verfüttert, dass man sich aber umsomehr von diesem Minimum entfernt, als man ein in seiner chemischen Konstitution differentes Eiweiss gibt." If this is strictly true, a protein wholly lacking in one or more cleavage products found in the tissues of an animal should be entirely inadequate for the construction of new body protein when fed alone. Gelatin, gliadin, and zein are in this class, yet all investigations with these proteins indicate that they are utilized as food by the animal even when fed as the sole source of nitrogen.[5] When fed in amounts corresponding to the fasting level of protein metabolism, a considerable portion of the nitrogen fails to appear in the urine as promptly as do forms known not to be of value to the animal. This phase of the subject will be further treated later when the results of Michaud are examined in some detail.

Michaud's method would appear in most respects to be a logical one for determining the value of a particular protein to an animal for purposes of repair of the tissue destroyed in endogenous metabolism. The plan is as follows: The animal is reduced to its lowest possible level of nitrogen elimination by feeding a nitrogen-free diet (starch, fat), and then an amount of nitrogen equivalent to the lowest daily output of which the animal is capable is fed in the form of the particular protein to be studied. The degree in which the animal utilizes the protein fed in replacing the tissue daily catabolized is taken as a measure of its value as a tissue-building material. The supposed efficiency of the method is based on the assumption that the animal will utilize the nitrogen presented to him for "repair" purposes as efficiently as it is possible to do so. Also that it is possible for an animal to take a given amount of nitrogen, and if its character is suitable, to convert it into one hundred per cent product, *i. e.*, body tissue. This assumption would seem to be supported by the experimental data available. Michaud found that when protein equivalent to the "protein minimum" was fed to dogs in the form of casein or dog tissues when the animals were metabolizing at their minimum level, there was no loss of nitrogen, which would seem to indicate a perfect utilization of protein at this low plane of intake.

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[3] Abderhalden: Zeitschrift für physiologische Chemie, 1909, lx, p. 425.

[4] Michaud: *Ibid.*, 1909, lix, p. 421.

[5] Compare Murlin: This journal, 1907, xix, p. 285; xx, p. 234; also Henriques: *Loc. cit.*; Abderhalden: Zeitschrift für physiologische Chemie, 1909, lx, p. 418.

The data presented in this paper is an outgrowth of a series of experiments undertaken two years ago, using essentially the method of Michaud, to compare the values of the protein mixtures of some of our most important grains as nutrients for the pig. It was hoped that specific differences would be shown by this method between grains in which the character of the protein mixture is known to differ so markedly as in the wheat, oat, and corn kernels. The plan of the experiments was as follows: the animal (pig) was fasted for two or three days with water and salts until it would take a starch solution readily, and then it was given fifty calories per kilo per day in the form of starch, together with a salt mixture having about the composition of the ash of the oat kernel with liberal excess of NaCl. The salt mixture was given in amount sufficient to make 4 per cent of the total ration. This mixture contained:

K_2O	15.5 per cent.
Fe_2O_3	3.0 " "
CaO	3.3 " "
MgO	5.0 " "
P_2O_5	25.0 " "
SO_3	4.0 " "
NaCl	44.2 " "

The salts and starch were treated with a small amount of boiling water to scald a part of the starch, the mass quickly stirred and cool water added in amount to form a soup which the pig could drink readily. This ration was continued with daily quantitative collections of the urine and feces. The creatinine and total nitrogen were determined daily. From the amount of nitrogen eliminated during five to ten days in the form of creatinine, an average was obtained from which the nitrogen resulting from endogenous metabolism was calculated, using the formula 5.5 × creatinine nitrogen equals nitrogen from endogenous metabolism.[6] The pig was then maintained on the nitrogen-free diet until the daily output of nitrogen in the urine became constant at about this level.

When the urinary nitrogen was entirely of endogenous origin for several days, as shown by a nearly constant ratio between the nitrogen as creatinine and the total nitrogen of between seventeen and nineteen to one hundred, the pig was considered to be ready for an experiment in nitrogen feeding. The grain to be studied was now introduced into the ration, and an isodynamic quantity of starch withdrawn. Since feeding a grain always leads to an increased excretion of nitrogen in the feces, it was assumed that part of the nitrogen is not digested, and accordingly the amount of grain fed was such that if 90 per cent of the nitrogen was digested and absorbed, the absorbed part would just equal the amount of nitrogen degraded daily in endogenous metabolism. Actually the increased nitrogen in the feces amounted to somewhat more than 10 per cent of the amount fed, so the amount absorbed was a little less than enough to meet the needs of the pig for repair. The results obtained were rather surprising. With the oat or corn kernel there was very little change in the nitrogen content of the urine or in the ratio of the creatinine N eliminated to the total nitrogen. These remained nearly the same as in the latter part of the period when a nitrogen-free diet was given. With wheat, a small rise in the nitrogen content of the urine was observed amounting to about 10 to 15 per cent of the amount fed. This difference was entirely too small to account for well-known differences in the quantitative relations among the cleavage products of the wheat proteins and those of animal tissues thus far studied.[7] The gluten of wheat makes up about 75 per cent of the total protein content of the grain, and yields about 40 per cent of its nitrogen as glutaminic acid, while all animal proteins thus far examined contain less than 17 per cent of this complex. It is scarcely possible that the mixture of cleavage products obtained from the wheat grain should be suitable for rearrangement into muscle proteins with an efficient utilization of nitrogen. In all of these experiments with individual grains the efficiency of the collection of the urine was tested by giving one or more doses of urea to see if the nitrogen administered in this form could be traced into the urine. Urea nitrogen was invariably recovered promptly to the extent of 85 to 90 per cent in the urine *in excess of the endogenous nitrogen*, and such excess of nitrogen in the urine was accompanied by a fall of the per cent of total nitrogen in the form of creatinine. These results led me to undertake a series of experiments with pigs in the feeding of:

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[6] McCollum: This journal, 1911, xxix, p. 210.

[7] Osborne: Ergebnisse der Physiologie, 1910, x, p. 47; also This journal, 1908, xxii, p. 433; 1908, xxiii, p. 81; 1909, xxiv, p. 161 and p. 437; **Abderhalden**: Zeitschrift für physiologische Chemie, 1907, li, p. 404 and p. 311.

1. Zein nitrogen equivalent to the endogenous urinary nitrogen.
2. Zein nitrogen equivalent to two to nine times the endogenous nitrogen.
3. Gelatin nitrogen equivalent to the endogenous urinary nitrogen.
4. Casein nitrogen equivalent to twelve or thirteen times the endogenous urinary nitrogen.

The plan of these experiments differed from those of Michaud in certain respects: Michaud took as the "Eiweissminimum" the sum of the urinary and fecal nitrogen excreted by the animal after a long period on a nitrogen-free diet. I have taken the nitrogen of the urine alone as an index to the amount of nitrogen to be fed, and have interpreted the results largely on changes in the nitrogen content of the urine resulting from feeding the forms of nitrogen studied. The nitrogen of the feces is not in the form of end products of metabolism, but represents losses which might be termed accidental in character: the cells abraded from the alimentary tract, the residues of the secretions which escape absorption, together with the bacterial growths for which these furnish a media, as well as undigested remains of food. These are all sources independent of the *essential tissue metabolism*. In experiments of this character we should deal specifically with the endogenous type of metabolism. The object should be to ascertain the influence of a particular protein on the repair of waste of an equivalent amount of tissue due to endogenous metabolism and not to attain a state of nitrogen equilibrium. The latter would require enough new growth to replace those losses of accidental nature. The nitrogen of the feces may serve as an approximate indication of the thoroughness of absorption provided adequate records are obtained of a fore and after period on a nitrogen-free diet.

The importance of taking the urinary nitrogen as the measure of the minimum protein metabolism is evident because of the vitiating influence of feeding nitrogen at too high a level in this class of work. It is evident that if we are to measure the efficiency of a particular protein by the excessive excretion of nitrogen during a feeding period, over the excretion on a nitrogen-free diet, it is imperative that the amount of nitrogen fed shall not exceed the urinary nitrogen of endogenous origin. If any excess over this amount is given, the animal is no longer limited by the one essential cleavage product present in smallest quantity. He is given some choice among a group of "building stones" larger than is necessary for repair.

Michaud makes no mention of giving his dogs an adequate supply of inorganic salts. In some of his feeding periods a certain amount of salts were carried by the protein mixture given, but when pure proteins were fed the dogs were apparently taking a nearly salt-free ration. In my own experiments a liberal supply of a salt mixture was given daily. The amount of water given was large enough to keep the volume of urine rather high. It was believed that this tended to a more prompt, uniform, and complete elimination of the end products of metabolism.

The investigation of this subject has extended over two years, and a very large amount of data has been collected. The tables here presented show representative protocols. All the data collected harmonized very well. The pigs used were from the Station herd, all were of the larger breeds, and were of the best-growing strains. Animals found to be infested with intestinal parasites were discarded.

An examination of Tables I and II shows that when a pig has been reduced by a long-continued, nitrogen-free diet, to a condition where the exogenous type of metabolism has probably entirely disappeared, the feeding of an amount of nitrogen as zein, closely approximating that derived daily as end products from endogenous metabolism, leads to a rise in the nitrogen content of the urine which is very much less than one would expect for a protein of this character. Although three amino acids are lacking, glycocoll, tryptophane, and lysin, all of general occurrence in animal tissues, it would seem evident that

TABLE I.

Experiment in feeding zein equivalent to the endogenous metabolism. Weight at beginning of experiment, 37 lb. (16.82 kgm.). Pig was placed in the cage March 11. Fed starch, salt mixture, and water during the following twenty days. No record of the nitrogen output until March 26. The endogenous metabolism of this animal, calculated from the creatinine output, is .863 gm. N per day (5.5 × .157).

Date.	N in food.	N in urine.	N in feces.	N as creatinine.	Per cent of total N as creatinine.
	gm.	gm.	gm.	gm.	
Mar. 26	0.0 (starch)	0.96	.24	.164	17.08
“ 27	0.0 “	0.94	.24	.143	15.21
“ 28	0.0 “	0.89	.24	.173	19.05
“ 29	0.0 “	0.86	.24	.165	19.18
“ 30	0.0 “	0.84	.24	.143[2]	17.02
“ 31	0.0 “	0.96	.24	.154	15.10
April 1	0.47 (zein + starch)	0.95[1]	.30	.166	17.46
“ 2	0.95 “	1.19	.30	.149	12.52
“ 3	0.96 “	0.96	.30	.144	15.00
“ 4	0.95 “	1.10	.30	.143	13.00
“ 5	0.95 “	1.08	.30	.168	15.37
“ 6	0.95 “	1.13	.30	.141	12.47
“ 7	0.95 “	0.90	.30	.139	15.44
“ 8	0.95 “	1.24	.30	.185	14.92
“ 9	0.47 “	1.01[3]	.30	.157	15.54
“ 10	0.0 (starch)	1.00	.28	.157	15.70
“ 11	0.0 “	0.83	.28	.154	18.55
“ 12	0.0 “	0.61	.28	.122	19.99
“ 13	0.0 “	0.81	.28	.133	16.42
“ 14	0.0 “	0.77	.28	.165	21.42
“ 15	1.0 (urea starch)	0.80	.28	.147	18.37
“ 16	1.0 “	1.27	.28	.157	12.36
“ 17	1.0 “	1.52	.28	.132	8.66
“ 18	0.50 “	1.59	.28	.156	9.81
“ 19	0.0 (starch)	1.21	.28	.162	13.39
“ 20	0.0 “	1.53	.28	.151	9.86
“ 21	0.0 “	1.23	.28	.141	11.47
“ 22	0.0 “	0.89	.28	.160	17.97

[1] Ave., .91 gm.

[2] Ave., .157 gm.

[3] Ave., 1.07 gm.

the animal has made use of a large part of the nitrogen of this protein. The ratio of creatinine nitrogen to total nitrogen falls but little from what it is on a nitrogen-free diet, which shows that the nitrogen of the urine is still derived principally from endogenous sources. The second starch period eliminates the possibility of a lag in the excretion of the zein nitrogen. The urea period following the second starch period shows how quickly the composition of the urine changes when a form of nitrogen useless to the animal is introduced. The total nitrogen rises at once, and with this goes a marked change in the per cent of the total nitrogen in the form of creatinine. Reference will be made later to the influence of similar amounts of gelatin nitrogen when administered to an animal under these conditions.

It has been customary with many workers in interpreting the data in experiments for determining the nutritive value of proteins, to judge from a plus or minus nitrogen balance as to whether the protein in question is sufficient or insufficient to maintain the animal in nitrogen equilibrium. We should go farther than this. Looked at in this way, the protocols shown in Tables I and II both show decided nitrogen deficits. If the nitrogen content of the feces is enough greater in the period when the protein is fed than it was during the last portion of the nitrogen-free period to indicate with certainty an incomplete digestion and absorption of the food protein, the *increase* in the fecal nitrogen should be considered as an index to the degree ot absorption. But knowing the amount of nitrogen absorbed, our judgment concerning its value for replacing the endogenous loss should be based entirely on changes observed in the urine, viz., on the increase, if any, in the total nitrogen, and the change in the ratio between the nitrogen as creatinine and the total nitrogen. Looked at from this standpoint, the tables give an accurate idea of the nutritive value of the nitrogen of zein for repair purposes.

During the nine days when zein was fed (Table I) the pig would have lost 8.19 gm. (9 × .91) of nitrogen if he had been kept on a starch diet. He was fed 7.59 gm. of nitrogen as zein, and his total output of nitrogen in the urine during the nine days from April 2 to April 10, inclusive, was 9.61 gm., which is only .16 gm. more per day than he would have excreted if no nitrogen had been given. The deficit of *urinary nitrogen* for the nine days was only 1.42 gm. It would seem therefore that the 7.59 gm. of nitrogen fed had replaced

TABLE II.

Experiment in feeding zein equivalent to the endogenous metabolism. Weight of pig at beginning of experiment, 158 lb. (71.8 kg.). December 6 to 10 pig was allowed to fast with water. December 11 to 17 fed 50 cal. per kilo as starch, with salt mixture and water. Urine not examined. During the four days' fast the animal was given 2 gm. of agar-agar daily to insure regular evacuations. The feces were collected from December 14 to 21 inclusive, no agar-agar being given. The feces for December 21 were marked off by a 15 gm. dose of $Ca_3(PO_4)_2$. The nitrogen content for this period was 6.16 gm. or .77 gm. per day. The remainder of the protocol is given in tabular form. The average daily creatinine output during the last four days of the period on a nitrogen-free diet was .475 gm. N. The endogenous metabolism of this pig calculated as 5.5 times the creatinine nitrogen was 2.61 gm. N.

Date.	N in food.	N in urine.	N in feces.	N as creatinine.	Per cent of N as creatinine.
Dec. 18	0.0[1]	2.59	.77	.48	18.64
" 19	0.0	2.61	.77	.44	17.01
" 20	0.0	2.66	.77	.50	18.94
" 21	0.0	2.58	.77	.48	18.83
" 22	2.62[2]	2.98	.72	.45	15.10
" 23	2.62	2.48	.72	.49	18.77
" 24	2.62	2.75	.72	.47	17.09
" 25	2.62	2.97	.72	.52	17.51
" 26	2.62	2.62	.72	.48	18.32
" 27	2.62	2.56	.72	.39	15.23
" 28	2.62	3.87	.72	.70	18.09
" 29	2.62	3.11	.72	.48	15.43
" 30	2.62	3.85	.72	.55	14.28
" 31	2.62	3.07	.72	.48	15.63
Jan. 1	1.31	2.37	.72	.39	16.45
" 2	0.0[1]	3.02	.72	.46	15.20
" 3	0.0	2.43	.68	.52	21.40
" 4	0.0	2.94	.68	.46	15.64
" 5	0.0	2.67	.68	.48	17.97
" 6	2.62[3]	2.58	.68	.48	18.60
" 7	2.62	4.90	.68	.48	9.79
" 8	0.0	5.04	.68	.52	10.31
" 9	0.0	3.01	.68	.47	15.61
" 10	0.0	2.70	.68	.48	17.40
" 11	0.0	2.66	.68	.48	18.04

[1] Starch (50 cal.). [2] Zein. [3] Urea.

6.77 gm. of this element lost through endogenous metabolism in this period.

An inspection of Table II shows that during the eleven days of zein feeding (December 23 to January 2, inclusive) the output of nitrogen in the urine was 32.67 gm., whereas the nitrogen output on a starch diet as calculated from the creatinine output would have been during the same time 28.8 gm. During this period 27.5 gm. N were fed as zein. The loss of nitrogen by the pig was thereby reduced from 28.8 to 3.8 gm. during the eleven days. Since, however, in the starch period following, .58 gm. of nitrogen was excreted in excess over what was to be expected, the loss should be considered about 4.5 gm. It would appear that, although zein is lacking in three amino acids, its efficiency as a repair material in cellular metabolism is quite high.

Table III shows the behavior of a pig on a ration supplying zein in quantities as high as about three times greater than the endogenous metabolism. If we consider the record beginning July 4 and ending with the experiment (thirty-four days), the nitrogen fed was 66.9. gm. The total elimination in the urine of the entire period was 76.7 gm. N, leaving a negative balance of 9.8 gm. N in the urine. The nitrogen content of the feces in the periods separated shows that the digestion was complete. The after-starch period was made long enough to bring the pig to the same level of nitrogen elimination as at the beginning of the feeding of zein. We must consider, however, that if the pig had received no protein during these thirty-four days (starch diet) the nitrogen elimination in the urine would have been about 37.4 gm. By feeding zein the loss of nitrogen from endogenous metabolism was reduced by 27.6 gm. That is, 27.6 gm. of this element degraded through tissue catabolism was replaced from the zein fed. Of the excessive nitrogen fed, however, none was stored as gain. The same care was given to the collection of the excreta as in the case of other animals in experiments in which the addition of a small amount of urea to the diet led to a prompt detection of a corresponding rise in the nitrogen excreted. The above difference is too large to be charged to experimental error.

It might be urged that the amount of zein fed in this case was too

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[8] Willcock and Hopkins: Journal of physiology, 1906–1907, xxxv, p. 88; Henriques: Zeitschrift für physiologische Chemie, 1909, lx. p. 105.

TABLE III.

Record of a pig on a ration supplying nitrogen in excess of the needs of the animal for repair, in which zein was the only source of protein. Weight of pig at beginning of experiment, 63.5 lb. (28.86 kgm.) Weight at end of experiment, 64.5 lb. (29.32 kgm.). The creatinine output during the first ten days on a starch diet averaged .201 gm. of nitrogen per day. From this the nitrogen equivalent to the endogenous metabolism of the animal was calculated to be 1.105 gm. (.201 × 5.5). The total nitrogen in the urine was not determined during the first five days. The nitrogen of the urine had not fallen to the lowest possible level at the end of ten days on a nitrogen-free diet, as shown by the value of the endogenous metabolism calculated from the creatinine output, and also by the fact that the nitrogen appearing as creatinine was still only about 16 per cent of the total. It was assumed, however, that the pig was sufficiently freed from the end products of metabolism for the purpose of this experiment, which was to determine whether an appreciable retention of nitrogen could be induced by feeding zein in excess of the "maintenance" requirements of the animal. All previous efforts to induce growth in animals by feeding zein as the sole source of protein have failed entirely,[8] but, as will appear later in this paper, the pig appears to be exceptionally efficient in the utilization of foodstuffs, so it seemed possible that more favorable results might be met with in the case of zein. The record given in the table begins with the sixth starch day.

Date.	N in food.	N in urine.	N in feces.	N in urine as creatinine.
June 29	0.0 (starch)	1.78	.59	.201
“ 30	0.0	1.70	.59	.189
July 1	0.0	1.42	.59	.194
“ 2	0.0	1.34	.59	.223
“ 3	0.0	1.29	.59	.210
“ 4	1.78 (zein)	1.20	.57	.204
“ 5	1.78	1.40	.57	.200
“ 6	1.78	2.19	.57	.197
“ 7	1.78	1.83	.57	.194
“ 8	1.78	1.73	.57	.174
“ 9	1.78	1.79	.57	.174
“ 10	1.78	1.95	.57	.214
“ 11	1.78	1.99	.57	.189
“ 12	1.78	2.13	.57	.199
“ 13	1.78	2.13	.57	.195
“ 14	2.23	2.04	.61	.214
“ 15	2.23	2.25	.61	.206
“ 16	2.23	2.23	.61	.185
“ 17	2.23	1.08	.61	.194
“ 18	2.23	2.43	.61	.192
“ 19	2.23	2.52	.61	.234
“ 20	2.23	2.38	.61	.201
“ 21	3.35	2.16	.66	.204
“ 22	3.35	2.80	.66	.182
“ 23	3.35	2.16	.66	.182
“ 24	3.35	3.78	.66	.195
“ 25	3.35	2.89	.66	.214
“ 26	3.35	2.98	.66	.200
“ 27	3.35	2.82	.66	.170
“ 28	3.35	3.21	.66	.196
“ 29	3.35	2.95	.66	.209
“ 30	3.35	3.14	.66	.228
“ 31	0.0	2.96	.66	.199
Aug. 1	0.0	2.64	.66	.204
“ 2	0.0	2.64	.66	.210
“ 3	0.0	1.61	.66	....
“ 4	0.0	1.62	.66	....
“ 5	0.0	1.08	.66	....
“ 6	0.0	1.29	.66	....

small in excess of the maintenance needs to be conducive to growth. It is true we know almost nothing of the influence of the plane of protein intake on the tendency of the growing animal to construct new body protein. Table IV is therefore presented, giving the record of a pig which took a relatively high protein diet in which zein furnished all the nitrogen.

TABLE IV.

Date.	N in food.	N in urine.	N in feces.	Date.	N in food.	N in urine.	N in feces.
Feb. 1	4.47	1.41	.74	Feb. 14	9.53	8.55	.74
" 2	4.47	2.35	.74	" 15	9.53	8.60	.74
" 3	4.47	3.27	.74	" 16	9.53	7.96	.74
" 4	6.59	4.35	.74	" 17	9.53	8.60	.74
" 5	8.82	4.23	.74	" 18	0.0	8.62	.74
" 6	9.24	8.46	.74	" 19	0.0	6.04	.74
" 7	9.53	8.28	.74	" 20	0.0	4.78	.74
" 8	9.53	8.44	.74	" 21	0.0	3.70	.74
" 9	9.53	8.13	.74	" 22	0.0	2.34	.64
" 10	9.53	8.23	.74	" 23	0.0	2.25	.64
" 11	9.53	8.18	.74	" 23	0.0	1.45	.64
" 12	9.53	7.43	.74	" 24	0.0	1.45	.64
" 13	9.53	8.23	.74	" 25	0.0	1.26	.64
Total					142.89	146.59	18.74

The pig from which the data given in Table IV was obtained, weighed 51 lb. (23.18 kgm.) at the beginning, and lost one pound during the experiment. He was fed a starch and salt diet (50 cal. per kilo) during an eleven-day fore period, during which the average N output in the form of creatinine was .19 gm. daily. Assuming that he would finally have established a ratio of 18.5 : 100 between creatinine N and total N, the endogenous metabolism of this pig was 1.04 gm. of nitrogen daily. This level was not reached in eleven days, the nitrogen content of the urine on the tenth and eleventh days of the fore period being 1.37 and 1.41 gm. respectively. However, it was believed that the animal was in a satisfactory condition for an experiment of this character. He was thereafter fed zein as indicated in the table.

The record shown in Table IV again shows a marked utilization of zein nitrogen in supplying material for the replacement of nitrogen lost through endogenous metabolism. Although the table as a whole shows a negative balance, it is by no means great enough to indicate a total lack of usefulness of zein nitrogen.

During the first seventeen days of the experiment the pig was fed 142.89 gm. of nitrogen. On account of the lag in the nitrogen excretion it is necessary to consider the feeding period and the starch period following, together. During the twenty-six days covered by these periods the nitrogen excreted in the urine was 146.59 gm. The total deficit was therefore 3.70 gm. If the pig had been kept on a starch diet, he would, judging from the creatinine output, have lost 27.04 gm. of nitrogen from endogenous metabolism. If none of the zein had been utilized by the pig, he should have excreted in his urine all of the nitrogen taken in this form together with the 27.04 gm. derived from endogenous sources. The nitrogen elimination in the urine should under these circumstances have been 142.89 + 27.04 = 169.93 gm. for the twenty-six days. The difference between this figure and the nitrogen found in the urine (169.93 − 146.59 = 23.34) we are to suppose was replaced from the zein fed. No new growth has been demonstrated in any case when zein has been the only protein supplied. These results with zein were so unexpected in character that it was thought desirable to make similar experiments with gelatin, using the pig, in order to compare the efficiency of this species with others in the utilization of this form of nitrogen. Gelatin has been used in nutrition studies more than any of the other "incomplete" proteins. In most of the recorded experiments it has seemed to me to be doubtful whether the animals were fed gelatin nitrogen at the same level as the endogenous metabolism, and that this fact would account for the lack of uniformity in the results of various observers as to the nutritive value of this substance. Accordingly six experiments were carried out on the same plan as used in the experiments recorded in Tables I and II. For presentation here the record is selected of a pig fed

TABLE V.

Experiment in feeding zein, and later gelatin equivalent to the endogenous metabolism. Weight of pig, 150.5 lb. at beginning, 152 lb. at end of experiment. The pig was fed starch (90 cal. per kilo.), a salt mixture, and water during a twenty-four-day period. A quantitative record was kept daily of the nitrogen content of the urine and feces and also of the creatinine output. The record presented in Table V begins with the twentieth day on a nitrogen-free diet.

Date.	N in food.	N in urine.	N in feces.	N as creatinine.	% of total N as creatinine.
Dec. 17	0.0	2.54	.94	.476	18.74
" 18	0.0	2.59	.94	.485	18.72
" 19	0.0	2.61	.94	.474	18.16
" 20	0.0	2.92	.94	.465	15.92
" 21	0.0	2.59	.94	.475	18.34
" 22	2.62 (zein)	1.98	1.06	.325	16.40
" 23	2.62	2.48	1.06	.516	20.80
" 24	2.62	2.75	1.06	.574	20.87
" 25	2.62	2.98	1.06	.425	14.26
" 26	2.62	2.62	1.06	.465	17.74
" 27	2.62	2.56	1.66	.473	18.47
" 28	2.62	3.87	1.06	.494	12.76
" 29	2.62	3.11	1.06	.481	15.46
" 30	2.62	3.85	1.06	.476	12.36
" 31	0.0 (starch)	3.07	1.06	.472	12.36
Jan. 1	0.0	2.37	1.06	.473	19.95
" 2	0.0	3.69	1.06	....	.....
" 3	0.0	2.43	1.06	.481	19.79
" 4	0.0	2.94	1.06	.471	16.00
" 5	2.62 (gelatin)	3.02	1.06	.474	15.69
" 6	2.62	3.15	1.04	.465	14.73
" 7	2.62	3.78	1.04	.473	12.51
" 8	2.62	4.32	1.04	.475	10.99
" 9	2.62	3.20	1.04	.472	14.75
" 10	2.62	5.57	1.04	.492	8.83
" 11	2.62	3.46	1.04	.476	13.75
" 12	2.62	3.24	1.04	.474	14.62
" 13	0.0 (starch)	3.02	1.04	....	.....
" 14	0.0	3.35	1.04	....	.....
" 15	0.0	2.60	1.04	....	.....
" 16	0.0	3.00	1.04	....	.....
" 17	0.0	2.40	1.04	....	.....

starch during a long fore period, then zein, followed by a starch period, then gelatin at the same level as zein, and lastly a starch period long enough to allow the pig to return to his endogenous level. The results were all in harmony, and showed an apparent utilization of between 50 and 60 per cent of the nitrogen given in this form for purposes of repair. The utilization of gelatin nitrogen fell in all cases far short of that from zein. Not more than 20 per cent of the nitrogen fed as zein has ever been traced into the urine in excess of the endogenous output, when the amount fed did not exceed 5.5 times the nitrogen daily eliminated by the animal as creatinine. When gelatin was fed in like amounts, between 40 and 50 per cent of the nitrogen given was promptly recovered in the urine in excess of what should have been found had a nitrogen-free diet been taken during the same period.

When considering experiments like that shown in Table IV, we are confronted by the fact that no very decided positive nitrogen balance has as yet been obtained in experiments where but one protein has been given. I have reported experiments in which an appreciable growth was secured in young rats fed a mixture of two proteins, together with carbohydrates, fats, and the necessary inorganic salts.[9] These are the most successful of any yet reported, with so simple a ration. The growth was in no case comparable to normal growth in the rat, and the high degree of uncertainty, and general unsatisfactoriness of the results, render this line of experimentation very discouraging as a means of throwing light on the chemical processes of metabolism. However, the importance of finding a mixture of pure chemical substances on which an animal will grow in a nearly normal way is very great, and warrants the expenditure of much effort in this direction.

In feeding rations compounded of relatively pure substances my attention has been repeatedly attracted by the marked differences exhibited by individuals of the same species, in their behavior toward such mixtures. Osborne and Mendel and Ferry have called attention to the same thing in their work.[10] This fact, together with a remarkable difference in the attitude of animals of different species toward rations made up of naturally occurring mixtures but derived from restricted sources, has been strongly emphasized in my own experience. Of twenty-four young rats ranging from 30 to 70 gm. in weight, when fed on wheat alone, but nine made gains of 15 gm. or more during the first three months. Eleven made no gains at all, but died in forty to sixty days, having suffered no appreciable change in weight during that time. Only two doubled their weight on this ration.[11] This did not happen in a single experiment, but in three experiments in three different years. In a similar trial with three young pigs of 50 pounds' weight in which the wheat kernel was the only food given, and the animals were kept away from vegetation, almost normal growth was observed during the first six months, and in the case of one animal with which the ration was continued nine months, a weight of 190 pounds was attained. This observation, together with the knowledge that a pig will readily take a sufficient quantity of a starch solution to cover his energy requirements, and will continue to do so for at least thirty-five days with no evidence of anorexia and no appreciable loss in weight, led me to try the pig on a mixture of casein, starch, salts, and water. The results were very gratifying and have convinced me that the pig is particularly suitable for this class of work. The records of two pigs kept in metabolism cages and fed this simple mixture, are shown in Tables VI and VII. It will be seen on inspecting Table VI that a pig of 48 pounds began to retain nitrogen at a remarkable rate, and continued to do so at a perfectly satisfactory rate during a period of thirty-six days. On February 13 it was noticed that the pig was not so eager to be let into the feeding stall as he usually was, but he entered of his own accord and ate all of his food. From that time on he grew more listless, but his appetite remained good enough to cause a complete consumption of food. The nitrogen output had been gradually increasing after the first two weeks. On the evening of the 16th, when he started to the feeding stall to eat, he fell and could not get up. He seemed to have lost the use of his legs, especially the hind legs. He was evidently in great pain, for he squealed loudly and trembled all over. The muscles were relaxed during this attack. After a quarter of an hour the evidence of pain disappeared, but the animal was still almost helpless. When helped to the trough he ate all of his food and was helped back again to the cage. The next day he seemed to rest easily

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[9] McCollum: This journal, 1909, xxv, p. 120.

[10] Osborne and Mendel and Ferry: Carnegie Institution bulletin 156, 1911.

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[11] All these rats if fed a normal ration should have attained weights of from 190 to 225 gm. in this period.

TABLE VI.

Experiments in feeding casein as the only source of protein. Weight of pig, 48 lb. (21.81 kgm.) at beginning, 55 lb. (25 kgm.) at end of experiment. The pig was allowed to go two days with water alone, and was then fed 100 cal. per kgm. as starch, an inorganic salt mixture with additional ground rock phosphate and casein as indicated in the table.

Date.	N in food.	N in urine.	N in feces.	Total output N.	N as creatinine.
	gm.		gm.	gm.	
Jan. 13	14.15	2.85	.60	3.45	.230
" 14	14.15	3.00	.60	3.60	.266
" 15	14.15	5.86	.60	6.46	.247
" 16	14.15	5.31	.60	5.91	.234
" 17	14.15	5.75	.60	6.35	.236
" 18	14.15	5.07	.60	5.67	.266
" 19	14.15	5.09	.60	5.69	.271
" 20	14.15	5.53	.60	6.13	.243
" 21	14.15	5.51	.60	6.11	.258
" 22	14.15	5.79	.60	6.39	.272
" 23	14.15	5.27	.60	5.87	.291
" 24	14.15	5.83	.60	6.43	.243
" 25	14.15	4.70	.60	5.30	.278
" 26	14.15	6.11	.60	6.71	.279
" 27	14.15	6.39	.60	6.99	.284
" 28	14.15	6.39	.60	6.99	.293
" 29	14.15	6.09	.60	6.69	.248
" 30	14.15	6.17	.60	6.77	.289
" 31	14.15	6.43	.60	7.03	.286
Feb. 1	14.15	5.90	.60	6.50	.293
" 2	7.10	7.93	.60	8.53	.324
" 3	7.10	8.70	.60	9.30	.285
" 4	10.66	9.04	.60	9.64	.277
" 5	14.21	7.67	.60	8.27	.320
" 6	14.21	7.50	.74	8.24	.302
" 7	14.21	9.00	.74	9.74	.284
" 8	14.21	8.04	.74	8.78	.287
" 9	14.21	8.39	.74	9.13	.325
" 10	14.21	8.31	.74	9.05	.290
" 11	14.21	8.16	.74	8.90	.311
" 12	14.21	7.77	.74	8.51	.328
" 13	14.21	9.57	.74	10.31	.307
" 14	14.21	9.66	.74	10.40	.272
" 15	14.21	9.92	.74	10.66	.330
" 16	14.21	9.75	.74	10.49	.355
" 17[1]	.....	9.82	.74	10.56	.312
....	478.38	248.27	23.28	271.55	....

[1] The pig died Feb. 17.

Nitrogen retained in the thirty-six days . . . . 206.83 gm.
Average creatinine N during the first five days . . . . . . .242 gm.
" " " " " last " " . . . . .315 gm.

Increase . . . . .073 gm.

and ate a portion of his food when assisted to the trough. When left alone he lay flat on his side with the legs extended but not rigid. The muscles were all relaxed and the reflexes were all present. When helped to his feet, he squealed constantly and shifted rapidly from one hind foot to the other, while turning around slowly all the time. When assisted to get off his feet, which he seemed unable to do himself, he assumed the posture described above, became quiet and was apparently much easier. He appeared no worse during the day, but at five o'clock, when urged to enter the cage, he fell again, squealing loudly, and died an hour later.

Autopsy revealed a marked congestion of the entire digestive tract, but much more pronounced in the posterior half. The colon was packed with putty-like feces. Conical necrotic areas were found on both kidneys. Dr. Hadley, Station Veterinarian, suggested acute poisoning, but an examination of the salt mixture failed to reveal the presence of any toxic metal, as was suspected. The casein used in this experiment was made by the method of Hammarsten, from separator skim milk from the dairy building of the Experiment Station, and all vessels used were glass. Nevertheless the experiment was repeated, using ash of whey instead of the salt mixture made up from reagent bottles. The same calcium phosphate was given as was used in the first experiment, — to give increased body to the feces. The second experiment was made with a pig which had been thirty-six days on a nitrogen-free diet. On the twenty-fourth day of casein feeding the pig fell into the same condition as the first, but was saved by promptly feeding whole milk and rolled oats. Food had to be introduced into the mouth at first, but after three days he was on his feet again, and after two weeks on the regular farm ration, in an open lot, during which time he gained 21 pounds in weight, the pig was fully recovered and was again placed on a starch ration and used in a long-continued experiment in low protein feeding.

It is scarcely warrantable to attribute the acute attacks of congestion which came upon these pigs, to the casein fed. Osborne and Mendel report having kept rats on a similar ration for a period of one hundred and sixty days without serious disturbances. The difficulty with these pigs is probably to be attributed to the peculiar conformation of the colon, and to impaction with feces of unfavorable character. This point is being further investigated.

TABLE VII.

Experiment in feeding casein as the only source of protein. Ration: casein, starch, ash of milk, NCl and water. Initial weight, 43.5 lb. and weight, 51 lb. This pig was kept on a starch diet (N free) from March 11 to April 16. The average creatinine output during the first ten days on starch diet was .191 gm. Only the last twelve of this period are included in the table. Began feeding casein April 16.

Average output of nitrogen as creatinine during the last twelve days on a starch diet, .189 gm. per day. Output during the last six days of casein feeding, .228 gm. per day.

Date.	N in food.	N in urine.	N in feces.	Total N output.	N as creatinine.
April 4	0.0	1.64	.34	1.98	.204
" 5	0.0	.76	.34	1.10	.188
" 6	0.0	1.24	.34	1.58	.174
" 7	0.0	1.07	.34	1.41	.200
" 8	0.0	1.12	.34	1.46	.189
" 9	0.0	1.01	.34	1.35	.205
" 10	0.0	1.12	.34	1.46	.193
" 11	0.0	1.10	.34	1.44	.207
" 12	0.0	1.02	.34	1.36	.184
" 13	0.0	1.21	.34	1.55	.191
" 14	0.0	1.02	.34	1.36	.192
" 15	0.0	.91	.34	1.25	....
" 16	6.86	.99	.39	1.38	....
" 17	13.72	2.22	.39	2.61	....
" 18	13.72	2.67	.39	3.06	....
" 19	13.72	5.85	.39	6.24	....
" 20	13.72	4.14	.39	4.53	....
" 21	13.72	4.77	.39	5.16	....
" 22	13.72	4.20	.39	4.59	....
" 23	6.86	5.28	.39	5.67	.219
" 24	13.72	5.41	.39	5.80	.203
" 25	6.86	4.29	.39	4.68	.234
" 26	13.72	11.33	.39	11.72	.212
" 27	10.97	9.46	.39	9.85	....
" 28	10.97	11.01	.39	11.40	....
" 29	10.97	8.34	.39	8.73	....
" 30	10.97	6.53	.39	6.92	....
May 1	10.97	5.66	.39	6.05	....
" 2	10.97	3.63	.39	4.02	....
" 3	10.97	5.53	.39	5.92	....
" 4	10.97	4.19	.39	4.58	.246
" 5	10.97	3.41	.39	3.80	.251
" 6	0.0	0.0	.39	.39	....
" 7	5.48	5.43	.39	5.82	.367
" 8	8.22	3.38	.39	3.77	.253
" 9	16.97	8.11	.39	8.50	.262
Total	259.74	139.05	13.44	152.49	....

Table VI shows a positive nitrogen balance of 206.8 gm. The stomach contained some liquid, and the intestine a considerable quantity of fecal matter, and the tissues of the animal some end products and partly metabolized nitrogenous substances. Experience with other animals leads me to believe that this pig's body contained about 25 gm. of nitrogen which would, if he had been placed on a starch diet, have appeared in the urine before the minimum level was reached. It would seem therefore that the pig had utilized for the construction of new body tissue about 175 gm. of nitrogen taken as casein. With this retention of nitrogen there was a gradual rise in the output of creatinine from .242 gm. per day during the first five days, to .315 gm. during the last five days.

The pig whose record is shown in Table VII had, when taken ill at the end of the experiment, eaten 259.74 gm. of nitrogen, and had excreted during the same time 152.49 gm. Since this pig was excreting only about 4 to 5 gm. of nitrogen daily in the urine, it is probable that he would have returned to the minimum level of nitrogen excretion, had he been placed on a starch diet, with a loss of about 15 gm. of nitrogen. This pig had retained and converted into body tissue about 80 to 85 gm. of casein nitrogen. It is unfortunate that the creatinine record was disturbed by the failure of the pig to eat or void any urine on May 6. This makes it impossible to arrive at a very satisfactory figure for creatinine at the end of the experiment. The average for the beginning was .189 gm. and for the last six days, after feeding casein, .228 gm. per day.

If we can judge from the change in the creatinine elimination, as to the body content of protoplasm, we must conclude that these pigs increased their metabolizing tissue by an amount equivalent to one fifth to one fourth on a diet containing but a single protein. This is without doubt the best evidence yet produced, of the *chemical* sufficiency of casein for both maintenance and growth. These experiments with casein greatly increase the value of the negative results obtained in the attempts to induce growth by feeding zein as the only protein.

Discussion of Results.

As before stated, the experiments reported in this paper were planned to give an answer to the question: Why is the procedure of reducing an animal to its lowest possible level of protein metabolism by feeding a starch diet, then feeding an amount of nitrogen in the form to be studied, equivalent to the endogenous metabolism and observing the excessive elimination of nitrogen in the urine, not a method for comparing the relative values of proteins as nutrients for that species? All of my observations have led to the belief that zein or gelatin when supplying the only source of nitrogen is in an important degree useful to the animal in replacing the nitrogen lost through endogenous metabolism. These "incomplete" proteins, when fed in liberal amounts, do not seem to supply all the complexes necessary for growth. The results of Michaud with gliadin are substantially in harmony with mine for zein. In his gliadin period Michaud fed 1.42 gm. of nitrogen daily. The average negative balance during nine days was .350 gm. N, equivalent to 24.64 per cent of the nitrogen fed. In his other gliadin periods the negative balances were between 15.05 and 36.33 per cent of the nitrogen fed. Murlin found gelatin to be utilized by dogs to the extent of 31 per cent of the nitrogen given. My results differ from his only in their greater uniformity, due to the fact that my animals were reduced to their endogenous level of metabolism in the fore period, whereas it seems to me very doubtful whether any of his were in this condition. Even with a liberal daily elimination of urine, I have not observed pigs to reach this level on a nitrogen-free diet in less than sixteen days.

The fact that certain proteins, lacking in one or more cleavage products known to be necessary to the formation of the proteins of the animal body, are of relatively high efficiency in preventing loss of body nitrogen due to endogenous metabolism, yet are insufficient for growth, forces one to the conclusion that the processes of replacing nitrogen degraded in cellular metabolism are not of the same character as the processes of growth. It seems also to be a necessary conclusion that the processes of cellular catabolism and repair do not represent a series of chemical changes involving the destruction and reconstruction of an entire protein molecule. This idea does not conflict with the theory of protein metabolism offered by Folin.[11] Osborne and Mendel[12] have recently shown that certain proteins may support an animal in nitrogen equilibrium for long periods and yet be unable to produce growth. My experience in feeding casein as the only protein has convinced me that a marked increase in the protein content of the body may result from taking a single protein in the food. The facts therefore lead to the belief that in order that an animal may grow, the food protein must supply complexes not necessary for the endogenous upkeep.

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[11] Folin: This journal, 1905, xiii, p. 117.

[12] Osborne and Mendel: *Loc. cit.*

It is interesting to correlate the results of these experiments with the findings of Folin [13] in his studies of the fate of creatin when fed to men on a very low protein diet. He was unable to trace the nitrogen of this complex into the urine under these conditions, and was led to conclude that creatin acts as a food and not as an end product of metabolism.

These fragments of evidence do not harmonize with the views of Michaud,[14] which have been endorsed by Aberhalden.[15] In fact, it seems rather surprising that Michaud should draw the conclusion quoted early in this paper, when he could trace but 15 to 36 per cent of the nitrogen of gliadin into the urine in his feeding experiments with this protein known to be lacking in one cleavage product, lysin, present in all animal proteins examined. According to his theory this protein should be without value to the animal, so far as its nitrogen is concerned, if fed alone.

SUMMARY OF CONCLUSIONS.

1. Attention is called to the fact that the results of experiments in feeding the mixture of proteins occurring in individual grains, in quantity equivalent to the lowest possible level of protein metabolism of which the animal is capable, do not indicate as wide differences in the nutritive values of the protein of the wheat, oat, and corn kernels as would be expected from the known chemical differences in these proteins.

2. Experiments are described in feeding zein and gelatin, two proteins which are "incomplete" chemically, in that they lack certain cleavage products known to be present in animal proteins. It is shown that the animal can utilize the nitrogen of zein very efficiently

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[13] FOLIN: Festschrift für Olaf Hammarsten, 1906.

[14] MICHAUD: *Loc. cit.*

[15] ABDERHALDEN: Zeitschrift für physiologische Chemie, 1909, lx, p. 425.

for repair of the losses due to endogenous or tissue metabolism. The average utilization of zein nitrogen for this purpose was about 80 per cent, for gelatin 50 to 60 per cent. No evidence was obtained of the formation of additional body tissue from zein, even when the latter was fed in great excess over the maintenance needs of the animal.

3. Experiments in feeding casein as the only protein resulted in increases of the body protein of 20 to 25 per cent. These are the most successful growing experiments yet reported in which but a single protein was fed.

4. The experimental data presented are shown not to harmonize with the most widely accepted theories concerning the mechanism of protein metabolism. The repair processes are shown to be of a different character from the processes of growth. The results of the work here presented are believed to indicate that the processes of cellular catabolism and repair do not involve the destruction and resynthesis of an entire protein molecule.

THE RELATION OF TONUS TO ANTIPERISTALSIS IN THE COLON.

By W. B. CANNON.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN Jacobi's incidental mention of antiperistalsis on the cat's colon, he noted that the waves started from a more or less broad constriction ring.[1] In the report of my observations on colonic antiperistalsis, made without knowledge of Jacobi's record, I stated that the waves began "at the nearest tonic constriction."[2] Elliott and Barclay-Smith also observed in various instances that the waves, both in the cat and the rat, were initiated "at the anal limit of a distended area," "from the upper limits of a ring of constriction," "from a deep constriction which formed and remained with slight oscillations as a starting point."[3] Fig. 6 in my paper and Figs. 2 and 3 in the paper by Elliott and Barclay-Smith represent clearly the waves passing away from the tonic ring[4] of origin. Although we thus reported our observations, none of us seems to have realized adequately the significance of the ring of tonic constriction as the source of antiperistalsis.

Two years ago I reported that by producing a tonic ring in the proximal colon exposed under warm normal salt solution, either by pinching the gut or by applying with a brush a weak solution of barium chloride, waves could be made to appear at will. By making the ring at the cæcum, repeated downward-running waves may be set going; by making a new ring now at the terminus of these waves, reversed waves appear and meet the downward waves progressively nearer the cæcum until only reversed waves are running; furthermore, a tonic ring made midway in the proximal colon has been seen giving rise to repeated waves which passed away in both directions.[5] These activities have been observed not only in the colon still receiving its normal blood supply, but also in the excised colon, tied full of fluid, and kept alive in warm oxygenated Ringer's solution. The waves have also been seen passing away from a tonic ring in the excised colon of the rabbit. The phenomena of the tonic ring are therefore of prime importance in explaining the occurrence of both peristalsis and antiperistalsis of the large intestine. In this paper I propose to examine further these phenomena, as well as some characteristics of the antiperistaltic waves, in an attempt to find the more intimate relations between these two activities of the gut.

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[1] Jacobi: Archiv für experimentelle Päthologie und Pharmakologie, 1890, xxvii, p. 147.

[2] Cannon: This journal, 1902, vi, p. 265.

[3] Elliott and Barclay-Smith: Journal of physiology, 1904, xxxi, pp. 280, 281, 284–285.

[4] The tonic state is conceived in this and the following paper as a persistent shortened condition of the musculature without any theory as to its being due to continued excitation. (See Biedermann, Archiv für die gesammte Physiologie, 1904, cii, p. 520.) The tonic ring is a region in which the persistent shortening is greater than elsewhere.

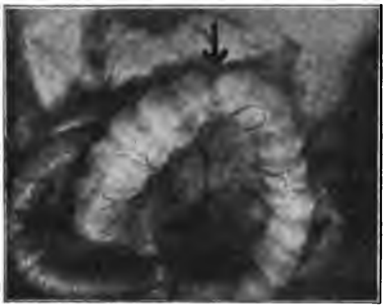

FIGURE 1. — Photograph of the colon of the cat, exposed under warm normal salt solution. A series of antiperistaltic waves are shown passing away from the tonus ring, which is marked by an arrow.

CHARACTERISTICS OF COLONIC ANTIPERISTALSIS.

The first characteristic of colonic antiperistalsis, which distinguishes it from peristalsis of the small intestine, is the absence of a region of inhibition projected before any moving constriction. As a result the constrictions continue passing over the proximal colon in a close series (Fig. 1), each succeeding ring never checking or interfering in any way with those already started and progressing before it.

The antiperistaltic waves, which run thus in a series, appear rhythmically. They must therefore start at a source that is rhythmically active. If an animal is etherized, its spinal cord destroyed below the brachial region, and the abdomen opened under warm salt solution, the colon can be directly watched. If the colon is inactive, but not wholly relaxed, it can be made to exhibit peristalsis by producing a tonic ring with barium chloride. The ring first contracts strongly; then, as it slightly relaxes, it can be seen pulsating rhythmically. Inspection of the colon under these circumstances shows that each pulsation sends away a moving constriction — a wave of peristalsis. The same phenomenon is observed when the colon is spontaneously active. The rhythmically beating tonic ring is therefore the source of the rhythmically recurring waves.

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[5] CANNON: This journal, 1909, xxiii, p. xxvii.

THE IMPORTANCE OF INTERNAL PRESSURE.

Antiperistalsis in the colon is dependent on a state of tension. The evidence for this statement is as follows. If a tube is tied into the colon and, as fluid is introduced, a tonic ring is made, antiperistaltic waves are usually started by the ring. If now the fluid is largely withdrawn, visible waves cease. Reintroducing the fluid starts them again. The observation which I made in 1901, that antiperistalsis begins whenever new material is pushed into the colon from the ileum, and Elliott and Barclay-Smith's method of starting the waves by injecting air or gruel, agree completely with other evidence that distention of the gut is the condition under which the waves originate. Indeed, Elliott and Barclay-Smith ascribe the appearance of the waves to distention.[6]

Mechanical extension is well recognized as a most efficient stimulus for exciting activity in smooth muscle. Sokoloff and Luchsinger, who observed the activity of the excised ureter through which normal salt solution was forced under varying pressures, reported that within wide limits the higher the pressure, the more frequent the pulsations, and expressed the view that a certain tension was the condition for the appearance of spontaneous contractions.[7] Guyon has reported similarly the contraction of the bladder as the result of distention to a requisite degree.[8] The manner in which the tonic neuromusculature of the excised stomach, which has been digesting, responds by contracting when distended by air has also been recorded.[9] Bayliss and Starling's observation that rhythmic contractions of the inactive small intestine of the cat appear when a balloon is inserted and inflated is further evidence for the same conclusion.[10] Of course, the attachment of a lever to a strip of smooth muscle (intestine, uterus) places the muscle in tension, and under the circumstances rhythmic contractions result and are readily recorded. Although strips of neuromusculature from the gut can contract spontaneously while resting without obvious tension in Ringer's solution,[11] yet the observations above cited point clearly to the importance of stretching as a highly efficient agency for evoking contractile response in smooth muscle.[12] Since smooth muscle surrounds hollow organs, which gradually fill and must be emptied, the value of distention as a stimulus is obvious.

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[6] ELLIOTT and BARCLAY-SMITH: *Loc. cit.*, p. 278.

[7] SOKOLOFF and LUCHSINGER: Archiv für die gesammte Physiologie, 1881, xxvi, p. 467.

THE IMPORTANCE OF THE TONIC STATE.

The extension of smooth muscle which evokes a contractile response must not, however, be merely the elongation of non-elastic substance. When smooth muscle is flaccid and toneless, extension calls forth no response. Only when the muscle is shortened and resilient, *i. e.*, in a state of tonus, does a pull result in a contraction. Thus the vagus nerves supply tonic impulses to the œsophagus and stomach. Immediately after the vagi are severed food gathers in the toneless œsophagus without causing any contractile reaction of the smooth muscle in the wall. Only after time has elapsed and a tonic state of the neuromusculature has been developed intrinsically, does the presence of food in the lower œsophagus again cause contraction.[13] Similarly impaired action of the rectum, the stomach, and the bladder has been observed immediately after severance of the nerves which

[8] GUYON: Comptes rendus de la Société de Biologie, 1900, lii, p. 712.

[9] CANNON: This journal, 1907, xx, p. 301.

[10] BAYLISS and STARLING: Journal of physiology, 1901, xxvi, p. 134.

[11] See MAGNUS: Archiv für die gesammte Physiologie, 1906, cxi, p. 153.

[12] The same conclusion has been drawn with reference to the cardiac muscle of invertebrates (see CARLSON: This journal, 1905, xii, p. 488).

[13] CANNON: This journal, 1907, xix, p. 443.

supply tonic influences to these organs.[14] Yet all these organs are capable of action when isolated from the central nervous system, but only after recovery of a tonic state. The tonic state is fundamental. I have repeatedly attempted to call forth rhythmic contractions, both in the atonic colon and in the atonic stomach, by distention, but without success. The tonic state is therefore quite as important as the internal pressure, — indeed, it is the condition for the existence of that pressure.

According to Schultz,[15] smooth muscle when in a short state is extended more readily by a moderate load than when in a long state and weighted with the same load. As Figs. 1 and 4 show, the tonic ring is a region contracted more than the neighboring regions, and ring *b* (Fig. 4), which is the pulsatile source of waves, is more contracted than ring *a*, which under the same pressure has ceased to pulsate (see p. 246). On the basis of Schultz's evidence we may assume, therefore, that at the tonic ring the neuromusculature may be in a condition especially favorable to distention by pressure. Furthermore, as already stated, since the ring is in tonic contraction, it will respond to extension by contraction.

THE RÔLE OF THE REFRACTORY PERIOD.

Magnus has brought forward evidence that during the period of shortening and the first part of the period of relaxation, the neuromusculature of the small intestine is relatively refractory both to electrical and to mechanical stimulation,[16] and that it only gradually, during relaxation, recovers full irritability. The neuromusculature of the stomach likewise, according to Ducceschi, passes through these same phases.[17] Refractoriness does not imply necessarily total abolition of excitability, it may mean only much diminished excitability as is the case during systole of the invertebrate heart.[18] As Fig. 2 shows, the neuromusculature of the colon is also refractory to stimulation while shortening, — either pinching close to the contracting ring, or pulling the ring sharply by traction on the lever, has no effect at this stage, although similar stimulation while the muscle is relaxing causes contraction. When the tonic ring begins to contract, therefore, in response to distention, it becomes refractory to the stimulus, *i. e.*, to the internal pressure. It begins again to be subject to this stimulus after having reached its most contracted state and partly relaxed, — *i. e.*, when again readily distensible. Now, on being distended by the internal pressure, the ring is stimulated, and as before responds in contraction. Thus, by the existence of the tonic state, by the presence of internal pressure, by the response of the tonic neuromusculature to the mechanical extension, and by its refractoriness during the period of shortening, the pulsations of the tonic ring can be accounted for.

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[14] ELLIOTT and BARCLAY-SMITH: Journal of physiology, 1904, xxxi, p. 289; CANNON: This journal, 1906, xvii, p. 441; ELLIOTT: Journal of physiology, 1907, xxxv, pp. 424, 425.

[15] SCHULTZ: Archiv für Physiologie, 1903, Supplement-Band, p. 25.

[16] MAGNUS: Archiv für die gesammte Physiologie, 1904, ciii, p. 540; 1906, cxi, p. 152.

[17] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, p. 170.

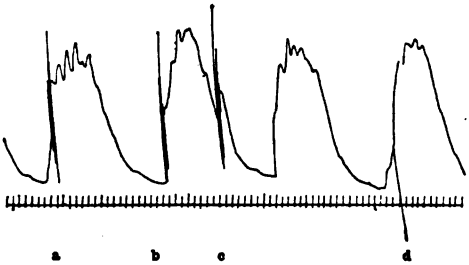

FIGURE 2. — Record showing the refractoriness of the large intestine to mechanical stimulation while contracting, and its irritability while relaxing. At *a*, *b*, *c* the stimulus was repeated pinching; at *d*, quick stretching.

THE TRANSMISSION OF THE ANTIPERISTALTIC WAVES.

The movement of a wave of constriction away from the pulsating tonic ring must now be regarded. In simple neuromuscular structures, especially in the functioning of the nerve net of invertebrates, v. Uexküll has so frequently observed that the state of excitation moves from an active to a less active region that he has declared the general law — "in a nerve net excitation flows from a place of higher tonus to a place of lower tonus," "from a contracted to a stretched muscle."[19] The departure of waves from a pulsating ring backwards, or forwards, or in both directions simultaneously, as described above, can thus be explained. It is not clear, however, that the spreading of the state of excitation into a freshly distended area, merely as a wave of internal change, is the manner by which the constrictions move. It is possible to invoke again in this progression of the constrictions the factors to which have been ascribed the pulsations of the tonic ring. Evidently any contraction of the tonic ring must increase internal pressure on neighboring parts of the intestinal wall. The muscles in these regions therefore are set in greater tension. As a result of the extra stretching, the muscle rings begin to contract and thus to distend the regions beyond, which in turn are stimulated. Thus, as a result of spreading distention, the passage of the constriction from the pulsating ring to inactive areas of the gut might be explained.

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[18] See CARLSON: This journal, 1906, xvi, p. 82.

[19] v. UEXKÜLL: Ergebnisse der Physiologie, 1904, iii^{2}, p. 4.

In this connection may be considered the reasons for the tonic ring normally sending away waves in only one direction, — *i. e.*, giving rise only to *antiperistalsis*. All instances of *peristalsis* have appeared, in my experience, when the colon has been injected with fluid to such an extent that a fluid content is present distal to the pulsating ring. Thus, when the waves pass away in both directions from the ring, the fluid character of the contents is the same in both directions. Under normal conditions, however, such is not the case. The contents become remarkably more viscous as they accumulate in the mid-region of the colon, indeed they may change to hard nodules. A tonic ring, with a fluid content on one side and a viscous mass on the other, would have different effects distally and proximally as it pulsated; through the viscous content it could not readily distend the wall and would therefore fail to discharge a wave distally; through the fluid content it would have its usual effect. Of course, if the distal content were moved by the contraction of the ring, the result would in all probability be different, for in that case there would be a moving stimulus. The observation of Elliott and Barclay-Smith — that in the rat the proximal colon, which is commonly worked over by antiperistaltic waves if the material it receives is soft and moist, exhibits the peristaltic reflex if the material is stiff and dry [20] — points clearly to the importance of consistency of the contents in determining the appearance of antiperistalsis.

A corollary from the foregoing discussion is that the less contracted region must itself still be in a condition to undergo distention, — it

[20] Elliott and Barclay-Smith: *Loc. cit.*, p. 285.

must, although not contracted, possess some degree of tonus in order to be in a state to respond to the extra pressure from within. If the gut is atonic and quite relaxed, the arousing of antiperistaltic waves from a pulsating ring is usually impossible. In my experience, also, waves do not move away from the ring if the gut expands sharply at the edge of the ring. Fig. 2 in Elliott and Barclay-Smith's paper[21] shows waves passing from a tonic ring to a region in less tonic contraction, but failing to pass to a region of full distention. In this case the distended and the contracted regions were separated by a rather abrupt change in the size of the lumen. The tube must taper from the less contracted part to the pulsating tonic ring or the pulsations of the ring will fail to discharge to the tube the moving constrictions.

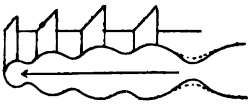

FIGURE 3.—Diagram of colonic antiperistalsis illustrating the regions refractory to stimulation (covered by horizontal lines), and irritable regions (covered by slanting lines). The rise of the slant lines indicates increase of irritability accompanying relaxation.

From the evidence that a refractory state attends the contractile period and that irritability is only gradually recovered during relaxation, the inferences are justified that the peristaltic contraction is itself a moving refractory phase of the neuromusculature, and that as it progresses it trails behind it a less irritable region. The condition that results in the colon is illustrated in the accompanying diagram of colonic antiperistalsis (Fig. 3). The narrowing at the right represents the pulsating tonic constriction, now contracted; the other narrowings represent waves moving away from the pulsating source. The horizontal lines extend in each instance, from left to right, over fibres just beginning to contract, fibres maximally contracted and fibres about one-third relaxed. The lines therefore lie above the parts of the muscular wall that are relatively refractory to stimulation. The slanted lines extend, left to right, from fibres about one-third relaxed to fibres wholly relaxed, — they reach, therefore, over regions in which irritability is being recovered. The upward slant of the lines indicates increasing susceptibility to stimulation as relaxation proceeds. Evidently the circumference fully relaxed is the region most ready to contract. This circumference in each instance is separated from the departing contracted region (that to the left in the diagram) by an area of diminished irritability. On the other hand, the most relaxed circumference is in each instance contiguous with the oncoming region of contraction. Thus, for example, if the circumference next the tonic ring has relaxed (as in Fig. 3), it is again most susceptible to an impulse coming to it from the pulsating source, or to increased pressure due to the pulsation. What is true of this relaxed circumference is true of each other fully relaxed band lying beyond — each is maximally irritable and most subject to the spread into it of the contraction phase existent on the side towards the tonic ring. Each is protected from being invaded by the contraction phase on the side away from the tonic ring by the intervening region of lessened irritability. Thus, if a contraction spreads, it necessarily spreads progressively from its origin into regions most ready to undergo contraction. And just as in the heart the pulsations of the sinus set the pace for contraction of the rest of the organ, so in the colon the pulsations of the ring determine the rate at which the waves shall appear.

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[21] ELLIOTT and BARCLAY-SMITH: *Loc. cit.*, p. 280.

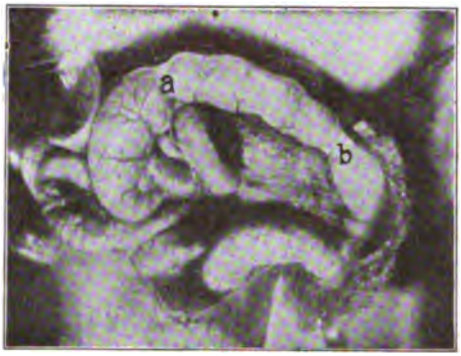

FIGURE 4. — Photograph showing the blocking of antiperistaltic waves started at tonic ring *b*, by the nearly relaxed ring *a*.

The observation previously noted (see p. 239) that waves from a new tonic ring will sweep away opposing waves from an older ring indicate that the pulsations are more rapid in a state of high tonus than they are later when the tonic state has somewhat diminished, and that the new ring therefore sets a faster pace than the older. This inference is justified by the decrease in the rate of pulsation with decrease in tonus. In one instance, as the ring was relaxing, the rate decreased, within five minutes, from 6 to 5 waves per minute.

A tonic constriction is itself refractory to the stimulus that comes to it in the form of a moving constriction. This fact was noted by Bayliss and Starling in the small intestine;[22] — my observations and those of Elliott and Barclay-Smith on the occurrence of antiperistaltic waves between the natural tonic constrictions of the distal colon also illustrate the definite boundaries set by the tonic state. The blocking of the waves started at tonic ring *b*, by the nearly relaxed ring *a* (Fig. 4), illustrates the refractoriness of the region in stronger tonus to influences from regions in weaker tonus.

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[22] BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 106.

THE RELATIONS OF TONUS, INTERNAL PRESSURE, AND PERISTALSIS.

The dependence of pulsations on an adjustment between locally increased tonus and the internal pressure is also illustrated in Fig. 4. The colon was filled with fluid; ring *b*, which was deep, was pulsating and sending forth waves; ring *a*, which had partially relaxed, no longer pulsated. The two rings were exposed to approximately the same internal pressure; this was an adequate stimulus for the deeper ring, but not for the less deep. Under such circumstances I have been able to renew rhythmic activity in the quiet ring merely by increasing the internal pressure. In all probability this is what occurs when new food enters the large intestine from the ileum and starts a fresh series of antiperistaltic waves. The new food distends the walls. A general contraction of the proximal colon follows, which presses the food towards the region of the tonic rings.[23] The inactive nearest ring then begins to pulsate in response to the increased internal pressure and thus to send waves backward towards the cæcum. Once started the waves can be artificially augmented by increase of internal pressure; thus shallow constrictions can be made much deeper by a series of slight momentary pressures on the gut, which cause repeated slight distentions of the wall where the waves are passing — an effect similar to that observed by Ducceschi, that up to a certain degree distention increases the contractions of the stomach.[24]

The precise relation between the degree of tonus and the internal pressure which results in effective rhythmic contraction is difficult to define. When a tonic ring is first made, either by pinching or by applying barium chloride, it is a deep and strong contraction. Only when it has to some extent relaxed does the normal rhythm appear and begin to send off waves.[25] Also, as already noted, withdrawal of fluid contents from an active colon causes immediate cessation of activity, which returns only as the contents are reintroduced. On the other hand, if the internal pressure is sufficiently increased, the waves moving along the gut will disappear, and can be seen again only when the distention is lessened. Sokoloff and Luchsinger report a similar experience in their experiments on distention of the ureter,[26] and likewise Jastreboff in his observations on the contractions of the rabbit's vagina.[27] Both the tonus and the distending force, therefore, can be too great for effective rhythmic action.

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[23] CANNON: This journal, 1902, vi, p. 268.

[24] DUCCESCHI: *Loc. cit.*, pp. 146, 149.

The Origin of the Tonic Rings.

From the foregoing evidence and discussion we can understand that, given in the colon the state of tonus and a locally increased tonic contraction, augmentation of internal pressure can explain the appearance of colonic antiperistalsis. The conditions for the establishment of the tonic rings, however, are still undetermined. It is probably significant that the tonic rings are not formed in the proximal colon, but only some distance from the cæcum. Elliott and Barclay-Smith were unable to demonstrate any motor innervation of the proximal colon. Possibly the tonic rings are formed only in that part of the colon which receives a motor nerve supply, and that the tonic state of that region stimulates the parts nearer the cæcum. This suggestion is supported by the observation that when the motor nerves of the distal colon are severed, the antiperistaltic waves become after a time very feeble.[28] Since, however, the rings do persist for a while after destruction of the spinal cord, they can be maintained by the gut itself. Henderson has called attention to the importance of the normal content of carbon dioxide in the blood for the preservation of the tone of intestinal smooth muscle; and by retaining the normal amount or by increasing it he has been able to induce antiperistalsis in the colon, as well as other movements of the canal.[29] That the proximal colon is the seat of the active fermentation which occurs in the large gut suggests the possibility that the tonic state may be in part at least due to the presence of intestinal gases.

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[25] At times, when the colon tapers towards the tonic ring, the region next the deepest tonus, somewhat less contracted, begins to pulsate before the ring pulsates. The tonus of this intermediate region can be regarded as adapted to the internal pressure. Compare the stomach (Cannon: This journal, 1911, xxix, p. 254).

[26] Sokoloff and Luchsinger: *Loc. cit.*, p. 466.

[27] Jastreboff: Archiv für Physiologie, 1884, p. 103.

[28] Elliott and Barclay-Smith: *Loc. cit.*, p. 287.

SUMMARY.

Antiperistaltic waves of the colon normally start at a tonic constriction in the gut, and they can be produced by making a tonic ring. They recur rhythmically, and originate in the rhythmic pulsations of the tonic ring.

These rhythmic pulsations are a response to the stretching of the tonically contracted muscle by internal pressure. Withdrawal of the colonic contents stops the pulsations, return of the contents restores them. If the muscle is not tonically contracted, internal pressure does not produce pulsations.

The tonic ring is especially subject to stimulation by distention. As the ring relaxes it pulsates less rapidly, and as it relaxes more it stops pulsating. Refractoriness to stimulation during contraction and the first part of relaxation explains the rhythmic response.

The waves pass away from the pulsating source probably as a result of stretching the relaxing muscular rings by increased internal pressure. As these waves are themselves moving refractory phases trailing regions of lessened irritability, they can only progress in a direction leading away from their source.

The precise relations between the state of tonus and the internal pressure which induce rhythmic response cannot yet be defined. The distending force, and the tonus also, may be too great or too slight to result in effective pulsations.

The conditions which originate the tonic state in the proximal colon are not yet definitely determined.

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[29] HENDERSON, Y.: This journal, 1909, xxiv, p 70.

THE NATURE OF GASTRIC PERISTALSIS.[1]

By W. B. CANNON.

[From the Laboratory of Physiology in the Harvard Medical School.]

THE characteristic contractile activities of the stomach, so long as gastric digestion persists, are the continuously repeated peristaltic waves running over the pyloric end, and the tonic contraction of the cardiac portion. In many respects the peristaltic waves of the stomach are like the antiperistaltic waves of the colon. Like the waves of the colon they follow one another in a series, without any extensive forerunning inhibition such as is seen in the dog's small intestine. Like those of the colon, also, the waves of the stomach are not abolished by a large dose of nicotine,[2] which destroys the typical peristalsis of the small intestine.[3] Furthermore, since the gastric waves recur with remarkably uniform rhythm, they, like the waves of the colon, must have a pulsating source. The factors explaining colonic antiperistalsis may be expected, therefore, to account for the gastric waves as well.

THE RELATIONS OF GASTRIC TONUS, INTRAGASTRIC PRESSURE, AND PERISTALSIS.

As the condition for the appearance of colonic antiperistalsis an important relation has been found to exist between the internal pressure and the tonic state of the neuromusculature.[4] In the stomach also the same factors are operative, as can be demonstrated in various ways.

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[1] A preliminary account of this investigation was presented at the meeting of the American Physiological Society, December, 1910. See Proceedings, This journal, 1911, xxvii, p. xii.

[2] CANNON: This journal, 1909, xxiii, p. xxvii.

[3] BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 115.

[4] CANNON: This journal, 1911, xxix, p. 241.

1. If a cat which has not recently taken food is etherized, its spinal cord destroyed below the brachial region, and its stomach exposed by opening the abdomen under warm normal salt solution, the stomach may be found moderately contracted (*i. e.*, in a tonic state), or relaxed and flaccid. In either case the organ will be inactive. If now fluid is introduced into the stomach, the effect will differ according to the status of the muscle. In the contracted stomach the introduced fluid encounters resistance, the pressure applied causes distention of the gastric walls, and peristaltic waves are at once started. In the relaxed stomach, on the other hand, the introduction of even large amounts of fluid usually has no effect — the walls simply retreat before the increasing content.

These observations show that tonus (*i. e.*, a shortened state) may be present in muscle without observable activity, that extension of atonic muscle does not produce activity, but that extension of tonic muscle results in the characteristic rhythm.

That distention of the stomach is a stimulus to its contractions has long been observed. Spallanzani noted that when a hen, starved for a day, was given nuts to eat, no movements of the stomach occurred so long as the organ contained only a few nuts, but that activity started promptly as soon as the stomach began to be full.[5] Hofmeister and Schütz have recorded the appearance of powerful contractions of the excised stomach of the dog when it was inflated with air and carbon dioxide.[6] And Ducceschi found that distention of the gastric walls caused the appearance of rhythmic contractions, and that within limits the amplitude of these contractions increased progressively as distention increased.[7] All these observations are in accord with results obtained by experimentally stretching the smooth muscle of various other hollow viscera.[8] In none of these instances, however, so far as I have been able to learn, was any attention paid to the condition of the neuromusculature when the stretching was performed. The point which I wish to emphasize is that the tension may be regarded as composed of two factors, the tonic state of the muscle and the internal pressure. Only when the muscle is shortened and resilient, *i. e.*, in a state of tonus, does distention or stretching, in my experience, result in contraction. The muscle, in other words, must first of all be in a condition to be *stretched* and not merely unresistingly elongated, before stretching can be properly regarded as the efficient stimulus for rhythmic contractions.

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[5] SPALLANZANI: Versuche über das Verdauungs-Geschäfte, translated from the Italian, Leipzig, 1785, p. 30.

[6] HOFMEISTER and SCHÜTZ: Archiv für experimentelle Pathologie und Pharmakologie, 1885, xx, p. 15.

[7] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, pp. 146, 149.

[8] See CANNON: This journal, 1911, xxix, p 240.

2. A second consideration indicating the importance of the tonic state for rhythmic activity is that during gastric digestion the stomach maintains its contractions with a considerable tonic tightening always existent. Many observers have recorded the pressures which prevail in the stomach during its activity — pressures which vary between 6 and 19 cm. of water in the cardiac end.[9] These persistent pressures measure the degree of tonus of the gastric muscle. The tension which is the favoring state for rhythmic activity is provided in the neuromusculature itself. The condition of the gastric wall while peristaltic waves are running is, therefore, as if the wall had been stretched by the contents.

3. Still further evidence of the importance of tonic contraction for rhythmic activity of the stomach is found in the observation that tonic and rhythmic contraction vary concomitantly in experimental conditions. (*a*) Stimulation of the vagus nerve is known to increase markedly the tonus of gastric muscle, and, with that, to augment the peristaltic contractions.[10] (*b*) Severance of the vagus nerves causes a temporary loss of tonus of œsophageal and gastric smooth muscle and, with that, an absence or marked weakness of peristalsis.[11] (*c*) Administering a small dose of adrenalin (1 : 10,000) abolishes intragastric pressure (which measures the muscular tonus); and simultaneously peristalsis ceases. Fig. 1 is a record of intragastric pressure

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[9] See v. PFUNGEN: Centralblatt für Physiologie, 1887, i, pp. 220, 275; MORITZ: Zeitschrift für Biologie, 1895, xxxii, pp. 356–358; SICK: Deutsches Archiv für klinische Medicin, 1906, lxxxviii, p. 190.

[10] See MAY, Journal of physiology, 1904, xxxi, p. 264; also protocol, p. 255, *infra*.

[11] CANNON: This journal, 1906, xvii, pp. 431, 432; 1907, xix, p. 441; DUCCESCHI: Archivio di fisiologia, 1910, viii, p. 586.

(lower curve), obtained by introducing a tube through the œsophagus into an active stomach filled with fluid, and of gastric peristalsis (upper curve), registered by means of a thread connecting the pyloric portion of the stomach with a writing lever. The animal had been etherized, its lower spinal cord pithed, and its abdomen opened under warm normal salt solution. As the record shows, peristaltic waves were not restored to the pyloric end of the stomach until nearly forty-five seconds after the pressure (the tonus) began to rise. (*d*) Artificial establishment of tonus in an excised stomach causes the appearance of peristalsis. A cat was etherized, the aorta severed, and the bloodless stomach removed. The stomach, tied at the pylorus, was slightly distended with warm 0.2 per cent hydrochloric acid, then tied at the cardia, and placed in a small dish containing 500 c.c. of warm oxygenated Ringer's solution. No change occurred. Now 2.5 c.c. of a 10 per cent solution of barium chloride, which causes tonic contraction of smooth muscle, were poured into the Ringer's solution. As the excised stomach became contracted, peristaltic waves began to pass towards the pylorus. Fig. 2 is a photograph of these waves. In one instance they continued thus with regularity for nearly a half-hour. The concomitant variation of tonic contraction and rhythmic activity in all these instances clearly indicates a causal relation between the two phenomena.

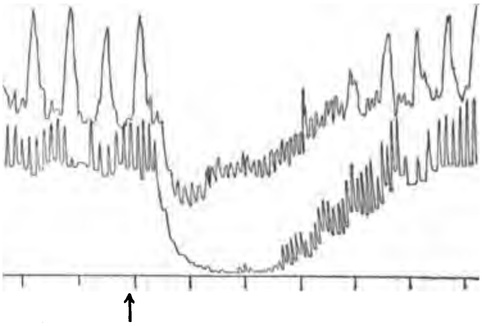

FIGURE 1. — Records of intragastric pressure and peristalsis of the pyloric end of the stomach. Peristalsis (upper curve) begins after pressure (lower curve) has begun to rise. Time, half-minutes.

All the evidence thus far adduced — (1) that distention of the inactive stomach causes contraction only when the muscle is tonically shortened, (2) that while the stomach is normally active a considerable intragastric pressure prevails (*i. e.*, the muscle is in sustained tension), and (3) that gastric peristalsis is augmented or weakened, is caused to appear or disappear, as the tension on the contents is increased or decreased — all this evidence points to two factors underlying rhythmic peristalsis of the stomach, the tonic state of the neuromusculature, and internal pressure which holds that neuromusculature in tension. As foregoing evidence has shown, the tension can be produced in two ways: by increasing the gastric contents and thus stretching the shortened musculature, or by such stimulation of the musculature that it is made to press tonically upon the contents. In the former case the two factors are separable; the musculature may exist in the shortened state without notable tension, and be put on a stretch only when internal pressure is applied. In the latter case the states of tonus and of internal pressure would be developed simultaneously. Now with tonus and internal pressure given as the elements for peristaltic activity, how can the peculiar manifestations of that activity in the stomach be explained?

THE SHAPE OF THE STOMACH AND THE PULSATING SOURCE OF PERISTALTIC WAVES.

There is evidence that the shape of the stomach is a primary factor in determining certain characteristics of gastric peristalsis. The stomach when first filled is roughly conical, — large in circumference at the cardiac end and progressively smaller as the pylorus is approached (see Fig. 2). If the contents are fluid or semifluid, and are subjected to the tension of the gastric musculature, the pressure throughout the contents (gravity aside) will be uniform. Every unit area of the wall will then be supporting the same pressure. Obviously a circumference of given width in the larger cardiac end will be subjected to greater total stress than a circumference of equal width in the smaller pyloric end. Since the forces in the inactive stomach are in equilibrium, however, the circular muscle of the cardiac end necessarily has to exert stronger tension than that in the pyloric end. And furthermore, since the muscular wall of the cardiac portion is thinner than that of the pyloric end, there are fewer muscle fibres in equal cross-sections. The greater circumference and the weaker musculature both tend to place the cardiac region at a disadvantage.

In a previous paper I have pointed out that it is difficult to define the exact relation between the degree of tonus and the internal pressure, which results in effective rhythmic contraction. The internal pressure may be too slight to evoke a response in the tonically contracted muscle, or it may be too great.[12] Ducceschi's observation that by causing intragastric pressure he could first rouse the inactive muscle into activity and that then by gradually increasing the pressure he could produce, after a maximum rhythmic response, an abolition of activity,[13] is in harmony with my observations on the colon. There is then possible a subliminal, an optimum and a supermaximal internal pressure for stimulating contractile response. On the basis of these observations we may assume that at first the muscles of the cardiac end are too much distended to respond, and that those of the pyloric end are too little distended. Between the large cardiac end and the small pyloric end, however, along the conically shaped organ, the relations of internal pressure and tonus will be intermediate. At some circumference in this region the relations will be such that the neuromusculature will respond by contraction. This circumference will be the first seat of the rhythmic pulsations which start the peristaltic waves.

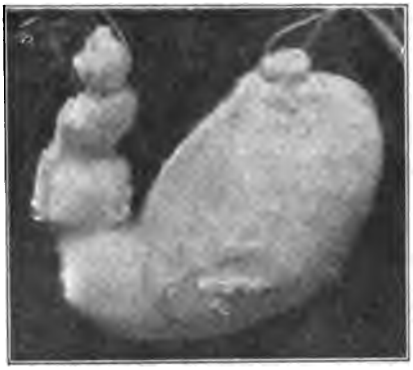

FIGURE 2. — Photograph of excised stomach in which peristaltic waves started when the stomach was tonically contracted by barium chloride.

Experimental support for the foregoing reasoning was secured by noting the effect on gastric peristalsis as intragastric pressure was increased and decreased.

A cat not fed for thirty-six hours, was etherized and given subcutaneously 1 c.c. of 1 per cent morphine sulphate; its spinal cord was pithed below the brachial region, and the abdomen opened under warm normal salt solution. At 11.25, 0.5 per cent HCl was introduced through a tube reaching through the œsophagus to the stomach. The empty stomach was slightly distended, but no peristalsis occurred. At 11.28, the cardiac end of the stomach was lifted out of the salt solution and quickly brushed with a 5 per cent $BaCl_2$ solution. At 11.32, shallow peristaltic waves began to pass over the stomach. In the interval, 11.34–39, more weak HCl was introduced and the waves disappeared, then about 30 c.c. withdrawn and they reappeared, again 30 c.c. introduced and they disappeared, 40 c.c. now withdrawn and waves, deeper than before, appeared; more HCl withdrawn until walls began to collapse, the waves disappeared. After the stomach was emptied, at 11.43, 50 c.c. of the 0.5 per cent HCl was introduced at 11.45, without starting peristalsis. At 11.48, 10 c.c. withdrawn, whereupon waves started at the pyloric end. Between 11.50 and 11.54, 40 c.c. were added to the contents very gradually, with the result that peristalsis was continued, but only at the end of the antrum. At 11.55, 45 c.c. withdrawn; peristalsis now began on the cardiac end and passed to the pylorus. At 12.05, manometer connected with the œsophageal tube, 20 c.c. introduced, pressure between 2.5 and 3.5 cm., peristalsis on pyloric end. At 12.10, 10 c.c. removed, no change, then 10 c.c. more removed, peristalsis started nearer cardiac end — pressure between 1.5 and 2.5. At 12.15, 40 c.c. introduced, peristalsis driven to end of antrum. Now the right vagus was stimulated, the stomach was strongly contracted especially in the pyloric end, and intragastric pressure rose above 22 cm. The pressure fell to 5–7 cm., and peristalsis, starting near the cardiac end, continued for nearly fifteen minutes. At 12.33, no peristalsis, pressure between 2 and 3 cm. At 12.35, 10 c.c. removed, pressure 1.5–2 cm., peristalsis reappeared. At 12.40, 100 c.c. introduced, peristalsis disappeared; right vagus stimulated, pressure rose to over 21 cm., then sank to 5–7 cm., peristalsis began near cardiac end and ran deep over antrum.

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[12] CANNON: This journal, 1911, xxix, p. 247.

[13] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, p. 147.

As the foregoing protocol and as other similar observations on the active exposed and on the active isolated stomach show, when intragastric pressure is increased without change in the state of the musculature, the place of origin of the waves is shifted towards the pylorus. And when the pressure is decreased by withdrawing some of the contents the place of origin is shifted towards the fundus. By the former procedure the over-stretched region (at the cardiac end) is extended, and the contracting circumference, having to meet a greater distending force, is moved to a region where the muscles are stronger and in a smaller ring. By the latter procedure precisely the opposite condition is brought about, — the muscle-rings towards the cardiac end, gradually less stretched beyond their responding power, begin to contract, and in consequence the source of the waves is moved farther towards the area of weakest musculature and largest circumference.

When the relation of tonus and internal pressure is such that contraction is stimulated, the contracting muscle-ring is, during the phase of shortening, refractory to stimulation. The refractory phase of smooth muscle with its local nervous system was indeed first demonstrated in the stomach.[14] The contracting muscle-ring displaces material, which is probably accommodated in the cardiac end where the weakest muscles are working against greatest obstacles. As the contracted circumference relaxes, however, the tonic pressure from the cardiac end again stretches the ring. Thus the contractions would be repeated rhythmically at this point, for the same reasons that have been given for the rhythmic response of the tonic ring in the colon.[15] The pulsatile action of the source of gastric peristaltic waves is indicated by the larger oscillations in the pressure curve of Fig. 1. The waves once started move onward continuously; their starting point, however, by its rhythmic contraction, rhythmically increases intragastric pressure.

THE TRANSMISSION OF THE PERISTALTIC WAVES.

The passage of peristaltic waves away from the pulsatile source must next be considered. These waves normally move only towards the pylorus. This direction is not taken, however, because the neuromusculature acts only in that direction. Again like the colon, the stomach may exhibit both peristalsis and anti-peristalsis. I have previously reported that a tonus ring made by applying barium chloride solution near the pylorus of a cat's stomach, will reverse the peristaltic waves over the antrum, and that excised frog's stomach, distended with normal salt solution, will show peristaltic or antiperistaltic waves according to which end of the organ is set in tonic constriction.[16] The failure of peristalsis to move from the pulsating source in the normal stomach backwards over the cardiac end is due, on the basis of the above reasoning, solely to the stretching of the musculature of that region beyond its ability to respond. Indeed, if it were not so stretched, it would itself be the pulsating source. In other words, the source is as near the fundus as the relation between tonus and internal pressure will permit; towards the fundus from that circumference the over-stretched muscle exerts merely a tonic grasp on the contents; towards the pylorus the muscle, not over-stretched, is capable of being roused to contraction.

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[14] See DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, pp. 165, 166.

[15] See CANNON: This journal, 1911, xxix, p. 243.

[16] CANNON: This journal, 1909, xxiii, p. xxvii.

The question as to how the contraction wave moves onward from its source is the same for the stomach as for the colon. The wave might progress as the result of an advancing ring of internal change, or it might progress because a ring of contraction would increase internal pressure on contiguous rings and thus call them into activity. In either case the rings just relaxing would be less irritable than rings already relaxed, and the effect, therefore, must be a wave moving steadily in one direction.[17]

Evidence as to which of the foregoing methods of transmission of the contraction wave prevails in the stomach was obtained by making, with aseptic precautions, incisions reaching to the submucous connective tissue and encircling the stomach. The myenteric plexus was thus completely severed. In different animals one, two, four, and six such complete interruptions were produced. When the animals had recovered from the operation (in all cases within four weeks), they were fed, and, after gastric digestion was well started, the stomach was exposed in the manner already described. Thus the movement of the peristaltic waves could be directly watched.

The trough of the wave was the part most easily followed, and its course could be observed best in stomachs with only one or two encircling incisions. In these cases waves at times passed over the organ as if no cut had been made. At other times there was only a slight hesitation at the ring of incision. Again, contraction occurred on the pyloric side of the cut and a wave started away just before the approaching wave arrived; the approaching wave then ended at the cut. Occasionally waves began at the cut without any perceptible waves appearing above it. In the case in which six rings of incision were made, peristaltic waves were clearly seen passing in monotonous rhythm over fully three-fourths of the stomach, without any disorganization, and with only occasional slight retardations at one or another of the rings.

Magnus has shown that the movements and the reflex of the small intestine persist after the mucosa and submucosa have been removed.[18] The submucous plexus, therefore, is probably not concerned in the motor functions of the canal. In the above experiments the continuity of the myenteric plexus was so far broken as to render it incapable of conducting. That peristaltic waves nevertheless can move with normal regularity over the stomach indicates that conduction by internal change (*i. e.*, the progress of a state of excitation in the nerve net), is not a necessary conclusion from the observed facts.

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[17] See CANNON: This journal, 1911, xxix, p. 245.

[18] MAGNUS: Archiv für die gesammte Physiologie, 1904, cii, p. 349.

A supposition accounting for the results obtained in the above experiments and in harmony with previous considerations is as follows. A contraction of the pulsating ring increases the pressure in the stomach. The increased pressure does not affect the contiguous muscle towards the fundus except to stretch it still more beyond its responding power. The muscle towards the pylorus, however, only contracts as internal pressure is increased. The rise of internal pressure resulting from a pulsation would therefore cause the contiguous muscle to be stretched and it would respond by contraction. Its contraction would in turn affect in the same manner the area lying immediately beyond, and thus the contraction would progress as a wave. It could not return on its path because, as already explained in relation to colonic antiperistalsis, the contraction wave trails behind itself an area of lessened irritability.[19] Thus severance of the myenteric plexus might have very little effect on peristalsis, for a wave approaching a ring of incision would distend the region beyond the ring quite as readily as under normal conditions, and the usual results would follow.

The foregoing explanation would require the pressure in the stomach to be greater as the pylorus is approached. Observations of intragastric pressure in different parts of the stomach show that such is the fact. In v. Pfungen's case of gastric fistula he found the pressure in the cardiac end 19 cm. of water, and that directly in front of the pylorus 162 cm. Moritz's records for the cardiac end varied between 6 and 8 cm., while in the pyloric end pressures rising to 38, 40, and even 60 cm. of water were obtained, with a usual range between 20 and 30 cm. Sick, using Moritz's method, found between 7 and 16 cm. of pressure in the cardiac end, contrasted with pressures from 25 to 42 cm. in the pyloric end.[20] These greater pressures in the pyloric end are doubtless due to the fact that the peristaltic waves become gradually deeper as the pyloric portion becomes gradually narrower. The contraction is increased, therefore, as the waves move into areas of thicker musculature contracting in smaller circumference. The greater mechanical stimulus required to induce contraction in this region compared with the cardiac region under the same conditions of tonus is provided naturally in the more powerful response of the stronger muscle. Thus, concomitantly, increased internal pressure (needed to stimulate contraction) and increased contraction (resulting in greater pressure) interact with and augment each other, as the waves move towards the pylorus.

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[19] CANNON: This journal, 1911, xxix, p. 246.

[20] See v. PFUNGEN, and MORITZ, and SICK: *Loc. cit.*

CONDITIONS WHEN THE MID-REGION BECOMES TUBULAR.

In 1898, I showed that as the stomach empties the mid-region becomes gradually narrowed into a tubular portion.[21] The waves then originate at the upper end of this gastric tube at a tonic ring separating the tube from the rounded cardiac sac. The ring forms a depression which has been repeatedly noted in X-ray photographs of the human stomach,[22] and is observable also in the exposed stomach of lower animals. This persistent constriction has been called the "incisura cardiaca." The activity of this deepened ring can best be understood in terms of the activity of the tonic ring in the colon. As the protocol (p. 255) records, and as numerous other cases have proved, the inactive stomach can be made to manifest peristalsis by producing with barium chloride a tonic ring in the cardiac end or in a circumference near the middle of the stomach. Since the stomach when first filled has a conical shape, the change to a tubular form in the mid-region involves greater contraction at the cardiac end than at the pyloric end (see Fig. 3). Because the cardiac incisure, at the extreme cardiac end of the tube, is therefore more contracted than any other part of the stomach, it, like the tonus ring in the colon, is probably more easily distended than any other part.[23] Distention by internal pressure, however, causes the ring to respond rhythmically. Each contraction sends off a wave towards the pylorus, and as the food is forced on into the intestine, the cardiac sac, by tonically pressing on its contents, provides more material for the waves, while helping to maintain the internal pressure required for the continuance of peristalsis. Only after the medium for distributing internal pressure — *i. e.*, the contents — has disappeared, does peristalsis normally cease.

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[21] CANNON: This journal, 1898, i, p. 371.

[22] See KAESTLE, RIEDER, and ROSENTHAL: Archives of the Röntgen ray, 1910, xv, pp. 21–24.

[23] The condition presented by the cardiac incisure clearly indicates the distinction to be drawn between muscle in a small ring (as in the antrum), and a small ring of tonically contracted muscle.

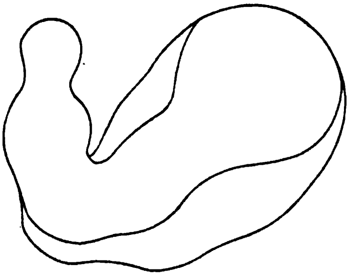

FIGURE 3. — Diagram showing that the change from the conical to the tubular shape of the stomach requires greater contraction at the cardiac end of the tube.

ORIGIN AND MAINTENANCE OF GASTRIC TONUS.

The important rôle of muscular tonus, developed in the foregoing discussion as a fundamental condition for gastric peristalsis, gives new meaning to vagus functions in the stomach. Vagus stimulation, as is well known, causes, after a momentary inhibition, a marked increase of tonus of the gastric muscle (see protocol, p. 256). With brief stimulation subsidence to the previous state may be rapid, but when a vagus nerve is repeatedly stimulated, the tonus increases more permanently after each stimulation, and in some instances may remain continuously high.[24] On the other hand, when both nerves are severed, the first effect is often total absence of activity.[25] Because peristalsis is associated with an augmentation of gastric tonus when a vagus nerve is stimulated, these nerves have been called the "motor nerves" of the stomach. From foregoing evidence, however, we may conclude that the function of the vagi is solely to set the gastric muscles in a tonic state, to make them exert a tension, so that they are as if stretched by the contents; and that the result of this condition is peristalsis.

The prime importance of the tonic state for the appearance of peristalsis is emphasized by development of a remarkable tonic state after the stomach is isolated from the central nervous system. As

[24] MAY: *Loc. cit.*, pp. 262, 264.

[25] CANNON: This journal, 1906, xvii, p. 431.

I pointed out in 1906, the diameter of the organ in the cat may under these circumstances be only 1.5 or 2 cm. — a smallness of size almost incredible.[26]

Whether the extrinsic nerves are present or not, therefore, the muscles of the gastric wall must be in tonus, and must be placed in tension by the contents before response will occur. In all probability the vagi normally adapt the size of the organ to the varying amount of food taken in. Thus, if the stomach were relaxed, these nerves might set the muscles in tension about a small amount of food which otherwise would not produce any tension whatever. If these nerves are severed, however, the intrinsic tonus which appears, compensates by rendering the stomach so contracted that, even if only a small amount is swallowed, the muscles are stretched, and peristaltic activities are at once started.

The question now arises as to the stage in the digestive process at which the vagus influences affect gastric tonus. I have previously stated that the severance of the vagi shortly before the ingestion of food is followed by inactivity for a considerable period. After digestion is well started, however, I have found, both by X-ray observations, and by inspection and records of intragastric pressure when the digesting stomach was exposed under warm salt solution, that the vagi can be severed without altering either the character of gastric peristalsis or the rate at which the stomach empties. The condition for peristaltic activity seems to be given to the stomach when the food is taken, and after that the stomach appears able to maintain that condition by itself.

That during the mastication and ingestion of food impulses pass down the vagi to the stomach, was proved by Pawlow's observations on the psychic secretion of gastric juice.[27] As already stated, repeated stimulation of the vagi results in an increased tonic state, which is much more persistent than that following a single stimulation. Since a tonic state is necessary for gastric peristalsis, and since peristalsis does not appear if the vagi are cut shortly before the ingestion of food, the inference is suggested that, just as there is psychic secretion, so likewise there is psychic tonus, aroused while food is being ingested.

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[26] CANNON: This journal, 1906, xvii, p. 432.

[27] PAWLOW: The work of the digestive glands, London, 1902, p. 50.

How gastric tonus, once established at the beginning of digestion, is maintained thereafter though the vagi be cut, is not yet determined. The presence of hydrochloric acid in the stomach, according to Edelmann, is the requisite stimulus for peristalsis.[28] Ducceschi had earlier noted that the acid applied to the cardiac end augmented the contractions,[29] and Hedblom and I observed that feeding acid food was attended by especially deep and rapid peristaltic waves.[30] Though there is this evidence of the concomitance of acid gastric contents and peristalsis, the conclusion that the relation is essential is not well supported. The empty stomach of a fasting animal is often found in tonic contraction and exhibits peristalsis as soon as distended, before acid secretion occurs; and powerful peristaltic waves may occur when the gastric contents are strongly alkaline.[31] Tonus, as a basis of peristalsis, is not, therefore, dependent on acid gastric contents.

The carbon dioxide content of the blood has been invoked by Henderson as the stimulus for peristalsis.[32] That increasing carbon dioxide in the blood is attended by increased tonus is easy to demonstrate. I have seen intragastric pressure rise from between 1 and 2 cm. of water to between 8.5 and 10 cm., and peristalsis change simultaneously from shallow to deep waves, as a result of adding a 40 cm. rubber tube to the tracheal cannula. Although the digestion of a meal is accompanied by an increase in the respiratory exchange, the carbon dioxide content of the blood during this time is probably not sufficiently different from its content at other times to account for the persistent tonus. Indeed the fasting stomach, as previously stated, may be found in strong tonic contraction.

Possibly the slow decrease in size (increase in tonic contraction) of the stomach, as the muscle contracts rhythmically, results from the "contraction remainder" of smooth muscle. This phenomenon, to which Schultz called special attention,[33] consists in the failure of the muscle to relax fully before the occurrence of another contraction. Evidently, if such a remainder were left as a heritage to each successive shortening, a process of adding would occur. The muscles would become more and more contracted — *i. e.*, the circumference of the stomach would be slowly diminished. The step-like rise in the pressure curve (Fig. 1) is explicable on the basis of the tonic state being built up of single contractions, the relaxation phases of which are not complete. Such a process can be observed in the excised stomach. I have tied the digesting stomach at the two ends, removed it from the body, placed it in warm oxygenated Ringer's solution containing 0.5 per cent barium chloride, introduced a glass tube which rose above the gastric level, and observed for nearly a half-hour peristaltic waves passing over the organ and the contents being gradually discharged as the capacity diminished. About 125 c.c. of material was thus gradually discharged. The stomach, therefore, seems to grow smaller by its own rhythmic contractions.

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[28] Edelmann: Dissertation (Russian) abstracted in the Jahresbericht über die Fortschritte der Physiologie, 1906, xv, p. 119.

[29] Ducceschi: Archivio per le scienze mediche, 1897, xxi, p. 154.

[30] Hedblom and Cannon: American journal of the medical sciences, 1909, cxxxviii, p. 518.

[31] Cannon: This journal, 1907, xx, p. 299.

[32] Henderson: This journal, 1909, xxiv, p. 71.

[33] Schultz: Archiv für Physiologie, 1903, Supplement-Band, p. 124; also Biedermann: Archiv für die gesammte Physiologie, 1904, cii, p. 520.

If a "contraction remainder" is to account for the gradual increase of the tonic state as digestion proceeds, it must act differently at different parts of the stomach. The mid-portion grows smaller much faster than the antrum (see Fig. 3). The process of adding to the contracted state must be therefore rapid and marked in the mid-portion, and only slight in the antrum. This differential effect is possibly accounted for by Ducceschi's observation that hydrochloric acid introduced into the antrum causes a diminution of the contractions in that region.[34] Thus the stronger musculature of the antrum would be held in check, while the rest of the stomach grew steadily smaller. The whole question of increasing tonus, however, must wait for further information regarding the nature of the tonic state in smooth muscle.

SUMMARY AND CONCLUSIONS.

Gastric peristalsis is like colonic antiperistalsis in consisting of serial waves starting at a pulsatile source and advancing without far-projected inhibition. Nicotine does not stop them.

Gastric peristalsis results from tension produced by internal pressure acting on the tonically shortened gastric musculature, — for (1) distention of the inactive stomach causes peristalsis when the musculature is tonically shortened, but not when it is relaxed; (2) considerable intragastric pressure (sustained tension) prevails in the stomach manifesting peristalsis; and (3) within limits peristalsis is augmented or weakened as intragastric pressure is experimentally increased or decreased. Tension can be produced by stretching tonically contracted muscle walls with increased contents, or by inducing tonus in the muscle.

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[34] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, p. 154.

The pulsatile source of the gastric waves has no fixed seat. The conical shape of the stomach permits a graded relation between internal pressure and the tonic musculature. The region that pulsates is that which is not over-distended or inadequately distended. By increasing internal pressure the over-distended region at the cardiac end is enlarged, and the source of the waves is moved towards the pylorus where the muscles have greater mechanical advantage.

The pulsations of the circumference where the waves start can be explained as the result of pressure causing contraction, and thus relative refractoriness to stimulation until relaxation is occurring. Then the ring is again stimulated as before.

The passage of peristaltic waves away from the source can be explained as the result of increased internal pressure, — for (1) increased internal pressure brings parts towards the pylorus into activity (see above); (2) internal pressure is greater as the pylorus is approached; and (3) the waves can move smoothly from the source to the pylorus when the continuity of the myenteric plexus is interrupted by many incisions encircling the stomach.

As the stomach empties, the cardiac incisure becomes the most contracted place. As such it pulsates most readily, and starts the peristaltic waves. Only when the medium for distributing internal pressure — *i. e.*, the contents — disappears, does peristalsis normally cease.

Tonus as an essential factor of gastric peristalsis is first given by vagus impulses and later maintained by the stomach itself, — for (1) severing the vagi before feeding results in inactivity, (2) severing the vagi after digestion is started causes no change in gastric movements or in the rate of discharge. Probably a psychic tonus is developed by the taking of food. The method of maintaining the tonic state locally as digestion proceeds, and of increasing it as the stomach empties, is not yet determined; it may be the result of a "contraction remainder" after each rhythmic pulsation.

The above results account for the functions of the vagi and the splanchnics. They are nerves which increase or decrease the tonus of gastric musculature, and thereby affect peristalsis. The absence of stomach movements, for example, in states of exhaustion, can be explained by the failure of vagus impulses; and in emotional states, by the presence of splanchnic impulses. Both conditions result in absence of gastric tonus.

THE RECEPTIVE RELAXATION OF THE STOMACH.[1]

By W. B. CANNON and C. W. LIEB.

[From the Laboratory of Physiology in the Harvard Medical School.]

IN 1898 Langley described the action of inhibitory fibres for the lower end of the œsophagus and the cardiac portion of the stomach.[2] With moderate electrical stimulation of a vagus nerve in the neck he observed relaxation of both these regions, followed by strong contraction when stimulation ceased. The function of inhibitory impulses for the lower œsophagus had previously been described by Kronecker and Meltzer.[3] They noted during repeated deglutition that the cardiac sphincter became more and more relaxed as the number of swallows increased, and that, after the passage of the swallowed material, the sphincter promptly contracted. This contraction was much more intense and lasted longer, the longer the series of swallowing movements that preceded.

The relaxation of the terminal sphincter of the œsophagus, as a peristaltic wave is driving a bolus towards it, is an excellent illustration of the general law that opposed muscles normally act, not in opposition, but in reciprocal co-operation — a law which Meltzer emphasized in this connection.[4]

That vagus innervation of the cardiac end of the stomach is similar to that of the cardiac sphincter received confirmation in an investigation by May, who found that with moderate stimulation the cardiac sac could be maintained in relaxation in some instances for sixty or seventy seconds, and that this inhibition was followed by an augmentor effect, indicated by increased tonus and peristaltic waves of greater amplitude than normal.[5]

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[1] The results of this investigation were reported at the meeting of the American Physiological Society in December, 1910. See the Proceedings, This journal, 1911, xxvii, p. xiii.

[2] Langley: Journal of physiology, 1898, xxiii, p. 407.

[3] Kronecker and Meltzer: Archiv für Physiologie, 1883, Supplement-Band, p. 358.

[4] Meltzer: Archiv für Physiologie, 1883, p. 215.

Since electrical stimulation of the cervical vagus affects in the same manner the lower œsophagus and the cardiac end of the stomach, it seemed probable that the act of deglutition might cause gastric relaxation, just as it causes relaxation of the cardia.

The importance of a relaxation of the stomach, especially of the large reservoir in the cardiac end, when food is swallowed, is obvious if the pressure in the active stomach is considered. Intragastric pressure has been reported in different animals and at different times as ranging from 7 to 19 cm. of water in the cardiac end, and from 25 to 162 cm. of water in the pyloric end.[6] These pressures are produced by the tonus of the gastric musculature. This tonic contraction may be regarded, therefore, like the tonic contraction of the cardiac sphincter, as an activity capable of opposing peristalsis of the œsophagus, and in the interests of organic economy preferably obviated when the œsophagus is driving material into the stomach.

That the cardia relaxes after swallowing and remains relaxed during repeated swallowing is another condition which renders inhibition of gastric tonus desirable, for if intragastric pressure remained high, and the cardia were patulous, the contents of the stomach might be pressed in large volume up the œsophagus after each act of deglutition.

The question of a receptive relaxation of the stomach when food is swallowed was tested by making, in cats completely anæsthetized, an œsophageal fistula, and, at various times after recovery from the operation, recording the pressure changes in the stomach. Through the lower opening of the fistula a soft smooth rubber catheter was introduced, bearing on its end a thin-rubber balloon, large enough, when filled, to occupy the entire cardiac end of the stomach. Into the end of the catheter, where the balloon was fastened, a piece of glass tubing was inserted. The apparatus was arranged so that the glass was situated within the cardiac sphincter, and thereby resisted any pressure which that sphincter might exercise. The outer end of the tube was connected with a float recorder—a cylinder 5.5 cm. internal diameter, with a small opening at the bottom to connect the catheter, and with a cork above supporting a writing lever. The balloon, the catheter, and part of the cylinder were filled with water. The cork, which nearly fitted the cylinder, and which was prevented by three inserted pins from rubbing the glass, rose and fell with the changes in water level, and affected the lever. The relatively large area of water in the cylinder permitted the recording of volume changes in the stomach without much alteration of intragastric pressure. Usually the water level in the cylinder was not more than 3 or 4 centimetres above the cardia.

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[5] May: Journal of physiology, 1904, xxxi, p. 262.

[6] See v. Pfungen: Centralblatt für Physiologie, 1887, i, pp. 220, 275; Moritz, Zeitschrift für Biologie, 1895, xxxii, pp. 356–358; Sick: Deutsches Archiv für klinische Medicin, 1906, lxxxviii, p. 190.

At times when the balloon was introduced the stomach was in a toneless condition, but usually a tonic state prevailed which was manifested by the supporting of a water pressure of several centimetres and the recording of rhythmic contractions. The phenomena now to be reported were studied only in cases in which tonicity was found.

When the animal, unanæsthetized, was resting quietly on the holder, and a regular record of rhythmic gastric contractions was being registered, a bit of raw beef was placed at the animal's nose. The meat was usually taken with avidity and quickly swallowed. It did not, of course, pass to the stomach, but emerged from the œsophagus at the upper fistula opening. A few seconds after the larynx rose in swallowing, intragastric pressure began to fall. As Fig. 1, which is a record of the volume changes of the balloon, shows, the drop in pressure is rapid, and is followed by only a slightly less rapid recovery of the former degree of tonus.

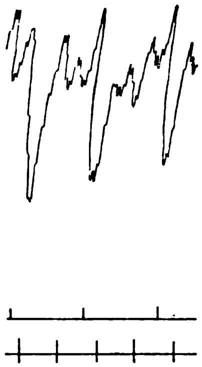

FIGURE 1.—Gastric relaxation after deglutition. The top line records changes of gastric volume; the middle line, acts of swallowing; the bottom line, time in half-minutes.

A record of the relaxations on a more rapidly revolving drum revealed that in some instances the drop in pressure began from two to two and five-tenths seconds, and in others from four and three-tenths to four and six-tenths seconds after the rise of the larynx. The lowest point of pressure was reached from six to ten seconds — in most cases about eight seconds — after the drop began. In 1898 Cannon and Moser reported that from ten to eleven seconds were required for a solid bolus to pass through the œsophagus of the cat.[7] It is clear from these figures that the stomach begins to relax a considerable period before the peristaltic wave has arrived at the cardia, indeed while the wave may still be moving through the cervical œsophagus, and that the greatest degree of relaxation is reached approximately when the bolus would be discharged into the stomach.

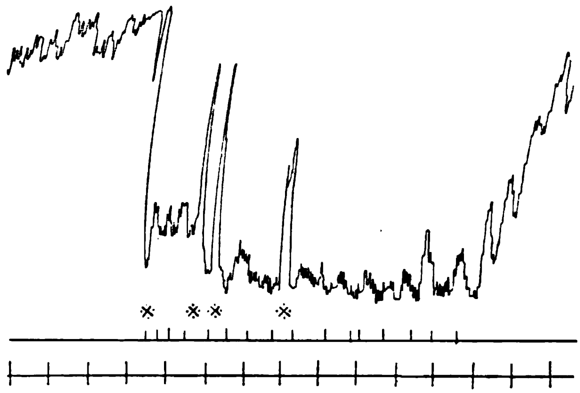

FIGURE 2. — Continued gastric relaxation with repeated deglutition. The asterisks indicate when the animal moved excitedly, causing quick changes of gastric volume. The middle line records acts of swallowing; the bottom line, time in half-minutes.

The increase of gastric volume, when the pressure upon the wall ranged between 3 and 4 cm. of water, varied in one instance between 8 and 10 c.c. In other instances the increase was not so great, and in one or two cases the record showed a relaxation which would accommodate only little more than 2 c.c. of fresh content.

By substituting a glass tube for the large glass cylinder, the pressure changes in the stomach could be observed with very little change in the volume of gastric contents. Intragastric pressure was observed by this means dropping almost to zero after swallowing.

The opposition which the tonicity of the gastric musculature might offer to œsophageal peristalsis is thus seen to be abolished almost precisely when the œsophagus is discharging food into the stomach;

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[7] CANNON and MOSER: This journal, 1898, i, p. 439.

and the relaxation of the gastric wall may be so great that as much as 8 or 10 c.c. of new contents may be received without much increase in the work of œsophageal muscles. This is an admirable instance, in the autonomic system, of the unidirectional working of the law of reciprocal innervation of antagonistic muscles.

As in the cardia, so in the stomach, repeated swallowing causes continued relaxation. Fig. 2 is a record of the persistent inhibition of gastric tonus resulting from repeated swallowing of bits of meat.

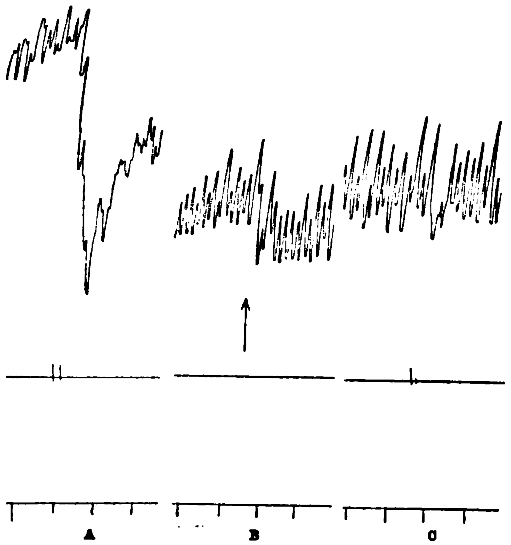

FIGURE 3. — Gastric relaxation after deglutition with right vagus cut (*A*), the moment of cutting the left vagus (*B*), and the failure of relaxation with both vagi cut (*C*).

When the animal became excited, the stomach was inhibited as a concomitant of the emotional condition, but recovery of the former state of tone was slower than recovery from the inhibition which follows deglutition. In Fig. 2 the sharp changes in the record of gastric volume (above the asterisks) were due to occasional excitement and movement of the animal. The subsequent lessening of tone is notable in each instance. The continued relaxation was probably at some moments in part due to the excitement.

The inhibition of gastric contraction which attends an emotional outbreak is due to impulses discharged by way of the splanchnic nerves.[8] The inhibition which follows deglutition is produced by way of the vagus nerves. This was proved by severing the right vagus nerve below the recurrent laryngeal in an aseptic operation, and later, after securing records of gastric relaxation after deglutition, severing the left vagus in the neck, whereupon the relaxation did not occur when the animal swallowed (see Fig. 3). That the relaxation normally begins a considerable time before the peristaltic wave in the œsophagus arrives at the stomach, indicates that the vagus impulses anticipate the œsophageal needs by a good margin.

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[8] CANNON: American journal of the medical sciences, 1909, cxxxvii, p. 485.

Since the receptive relaxation of the stomach is a reflex mediated through the vagus nerves as a result of the act of swallowing, it is different from the adaptation of gastric capacity to increasing contents, which Kelling has described;[9] and it is also different from the relaxation of the cardiac end of the excised stomach, reported by Sick and Tedesco.[10] In both these instances intragastric pressure does not fall; it merely fails within wide limits to become markedly increased as more contents are introduced.

Through the kindness of Mr. A. L. Washburn, who had accustomed himself to the presence of a balloon in the cardiac end of his stomach and a tube in his œsophagus, we were enabled to test the receptive relaxation of the human stomach after deglutition. The record of intragastric pressure showed a distinct drop after each act of swallowing.

The relaxation which occurs in the stomach is probably restricted to the cardiac end. The cardiac sac is the part of the stomach that varies most in capacity as the stomach is filled and emptied; it is the part that in both Langley's and May's observations was made to relax by electrical stimulation of the vagus in the neck; and it is the part in which the recording balloon rested in the experiments here described.

SUMMARY.

If the gastric wall is in tonic contraction, it relaxes after an act of deglutition. Intragastric pressure then falls nearly to zero, and the capacity of the stomach is readily enlarged. The lowest point of intragastric pressure is reached at approximately the time when a bolus is delivered into the stomach by the œsophagus. After the relaxation, which requires about ten seconds for its full development, there is a rapid recovery of the former tonicity.

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[9] KELLING: Zeitschrift für Biologie, 1903, xliv, p. 234.

[10] SICK and TEDESCO: Deutsches Archiv für klinische Medicin, 1907, xcii, p. 439.

Repeated swallowing causes continued inhibition of gastric tonus.

This receptive relaxation of the stomach is probably confined to the cardiac end. It is an instance of reciprocal innervation of antagonistic muscles, and is controlled reflexly by the vagus nerves.

THE EFFECTS OF ASPHYXIA, HYPERPNŒA, AND SENSORY STIMULATION ON ADRENAL SECRETION.

By W. B. CANNON and R. G. HOSKINS.

[From the Laboratory of Physiology in the Harvard Medical School.]

IN order that experimental results shall be reliable, it is necessary that the effects of common experimental conditions themselves shall be understood. A failure to regard these immediate effects of interference with organic processes has been a noteworthy source of error in the history of experimental biology. With the purpose of learning whether the artificial production of asphyxia, or the presence of hyperpnœa, or the stimulation of sensory nerves would affect the secretion of epinephrin, the present investigation was undertaken.

Since the procedures utilized required the use of a general anæsthetic, one must be employed which is not attended by increased adrenal activity. Without studying the influence of various general anæsthetics on adrenal secretion, — which will be the aim of another research, — we followed in the main the procedure described by Elliott[1] and found that urethane dissolved in a small amount of water and given, 2 gm. per kilo body weight, by stomach, to cats and rabbits, produced satisfactory general anæsthesia, free from any stage of excitement which might stimulate the adrenal glands, and in fact unattended by any increase of adrenal secretion demonstrable by our tests.

The method of testing for epinephrin in the blood was the modification of the intestinal strip method suggested by Hoskins,[2] — the use of segments of rabbit intestine, suspended lengthwise in a glass cylinder through which oxygen was passed. The segment, when not surrounded by the blood to be tested, was bathed in Ringer's solution

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[1] Elliott: Journal of physiology, 1905, xxxii, p. 448.

[2] See Hoskins: Journal of pharmacology and experimental therapeutics, 1911, iii, p. 95.

at body temperature. If a rabbit is anæsthetized with urethane, it will supply the highly sensitive fresh segments as they are needed. The animals experimented on were cats. Rabbit's intestine was proved to be quite as sensitive to a minute amount of adrenalin in cat blood as in rabbit blood. The innocuousness of cat blood for the rabbit intestine is shown by the prolonged rhythmic contraction of the rabbit intestine in that blood. The blood to be tested was taken before and after the experimental procedures by catheterizing the inferior vena cava, as described by Cannon and de la Paz.[3] Care was taken in all instances to insert the catheter to the same point anterior to the entrance of the adrenal veins, and to place the catheter opening (which was at one side) always in the same position relative to the wall of the vein.

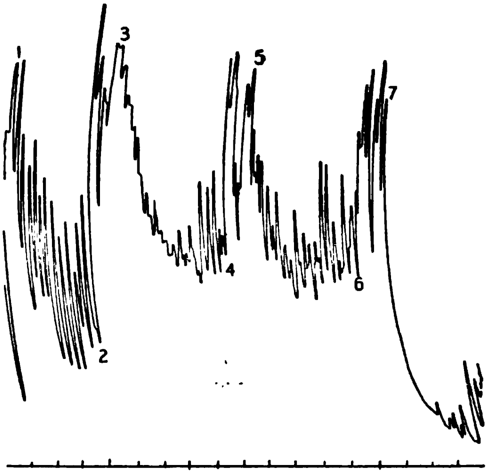

FIGURE 1. — Effect of asphyxia on adrenal secretion. At 1 normal vena cava blood applied, at 2 removed. At 3 normal blood from femoral vein applied, at 4 removed. At 5 blood from femoral vein after asphyxia applied, at 6 removed. At 7 blood from the vena cava after asphyxia applied. Time, half-minutes.

The effect of asphyxia. — The effect of asphyxia was tested by comparing the action of blood, taken as nearly simultaneously as possible from the vena cava and the femoral vein before asphyxia, with blood taken from the same sources after asphyxia had been produced. The femoral venous blood after passing the systemic capillaries thus acted as a standard for the same blood after receiving the contribution of the adrenal veins. Asphyxia was caused by covering the tracheal cannula until respiration became labored and slow, but capable of recovery when air was admitted. It may be regarded, therefore, as not extreme.

The results of the degree of asphyxia above described are shown graphically in Fig. 1. Blood taken from the vena cava and from the femoral vein before asphyxia ("normal") failed to cause inhibition of the contractions. Blood taken from the femoral vein after asphyxia produced almost the same effect as blood from the same vein before; asphyxia therefore had wrought no change demonstrable in the general venous flow. Blood taken from the vena cava after asphyxia had, on the contrary, an effect markedly unlike blood from the same region before, — it caused the typical inhibition which indicates the presence of adrenal secretion.

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[3] CANNON and DE LA PAZ: This journal, 1911, xxviii, p. 64.

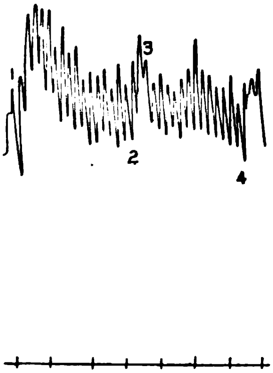

FIGURE 2. — Failure of hyperpnœa to cause increased adrenal secretion. At 1 normal blood from vena cava applied, at 2 removed; at 3 blood from vena cava after hyperpnœa applied, at 4 removed. Time, half-minutes.

This positive result might suggest that the comparison of both femoral and vena cava blood under each condition was unnecessary, and that a comparison merely of vena cava blood before and after asphyxia would be sufficient. Positive results were indeed thus secured, but they occurred even when the adrenal glands were carefully removed and extreme asphyxia (*i. e.*, stoppage of respiration) was induced. That the blood may contain in extreme asphyxia a substance or substances capable of causing inhibition of intestinal contractions was thus demonstrated. In one instance, after the blood was proved free from epinephrin, the aorta and vena cava were tied close below the diaphragm, and the carotids were tied about midway in the neck. Extreme asphyxia was produced (lasting five minutes). Blood now taken from the heart caused marked inhibition of the beating intestinal segment. Probably, therefore, the inhibitory action of blood taken from an animal when extremely asphyxiated cannot be due to epinephrin alone.

That the positive result obtained in moderate asphyxia, on the other hand, is not attributable to other agencies in the blood than epinephrin, is indicated by the failure of asphyxial femoral blood to cause inhibition, while vena cava blood, taken almost simultaneously, brought about immediate relaxation of the muscle.

The conclusion is justified, therefore, that asphyxia results in increased secretion of the adrenal glands.

The effect of hyperpnœa. — It is known that some bodily conditions, such as rapid heart beat and glycosuria, may occur during acapnia as well as during certain stages of asphyxia.[4] It was possible, therefore, that hyperpnœa, like asphyxia, would be accompanied by adrenal secretion.

Hyperpnœa was caused by opening the chest of the anæsthetized animal along the mid-sternal line, and holding the chest walls apart while the lungs were inflated in rapid repetition by means of bellows. The air forced into the lungs was permitted to escape quickly through an opening in the trachea. The method is only slightly modified from that used by Henderson in producing acapnia. Artificial respiration was continued in some instances for five minutes, and, in the instance which gave the record reproduced in Fig. 2, for ten minutes. Such artificial respiration would be followed by apnœa lasting for more than a minute.

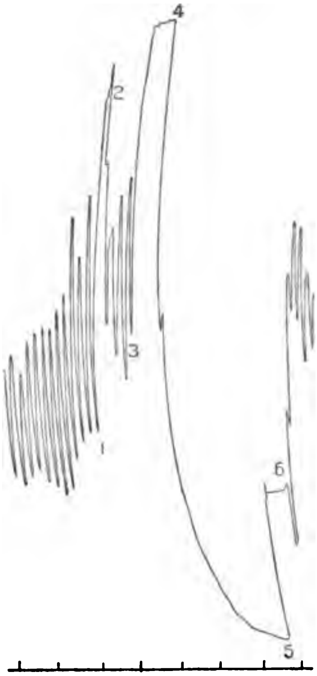

FIGURE 3. — Effect of sensory stimulation on adrenal secretion. Intestinal segment beating in normal vena cava blood, removed at 1 and renewed at 2. At 3 normal blood removed. At 4 vena cava blood after sensory stimulation applied, at 5 removed. At 6 Ringer's solution applied. Time, half-minutes.

As Fig. 2 indicates, the hyperpnœa caused by artificial respiration is not attended by any increase of adrenal secretion — the blood before and after the process has almost the same effect on the intestinal neuromusculature. In other instances than that represented in Fig. 2 the blood after hyperpnœa caused less relaxation than normal blood from the same animal, when applied after Ringer's solution to the beating intestinal segment. The suggestion might be offered that the greater oxygen content of the blood would favor more rapid oxidation of epinephrin, and that thus an increased secretion might be masked. That this explanation is improbable is indicated by the persistence of epinephrin in the blood when pure oxygen is permitted to bubble through it for much longer than ten minutes. It is altogether likely, therefore, that hyperpnœa, to a degree resulting in acapnia, is not attended by increased adrenal secretion.

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[4] See HENDERSON: This journal, 1908, xxi, p. 146; HENDERSON and UNDERHILL: This journal, 1911, xxviii, p. 287.

The effect of sensory stimulation. — Stimulation of sensory fibres in one of the larger nerve trunks is known to result in discharges along sympathetic paths. Thus such stimulation causes dilatation of the pupils, inhibition of gastric peristalsis and gastric secretion, and contraction of arterioles. Since the adrenal glands are stimulated to activity by sympathetic impulses, it was possible that they would be affected like some of the other organs innervated by the sympathetic system, and secrete in greater abundance when sensory nerves were irritated.

The sensory stimulus, applied to the sciatic nerve of the anæsthetized animal, was a tetanizing current which was increased in strength as the period of stimulation progressed, so that the pupils were kept dilated. Probably the stimulus used was much stronger than would be necessary to obtain a positive result in the absence of urethane, for Elliott found that urethane markedly lessens conductivity or irritability of visceral nerve fibres.[5] In different instances the stimulation lasted from three to six minutes. Throughout the period there was marked hyperpnœa.

As Fig. 3 shows, the normal blood, removed from the vena cava before stimulation, caused no inhibition of the beating segment, whereas that removed afterwards produced a marked relaxation. Since hyperpnœa does not result in an augmented adrenal activity, we may conclude that the adrenal glands are affected through nervous channels when a sensory trunk is strongly excited, and that under these circumstances they pour into the blood stream an increased amount of secretion.

The similarity between surgical shock and the condition of an animal after removal of the adrenal glands suggests that possibly in surgical shock the injury to large nerve trunks may discharge the adrenal glands to such a degree that they are unable to continue their normal functioning. Hesitating to add another to the already numerous theories of surgical shock, we merely wish to point out that since sensory stimulation causes increased adrenal secretion, adrenal fatigue or exhaustion may reasonably be regarded as an element in the complex.

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[5] ELLIOTT: *Loc. cit.*, p. 448.

EMOTIONAL GLYCOSURIA.[1]

By W. B. CANNON, A. T. SHOHL, and W. S. WRIGHT.

[From the Laboratory of Physiology in the Harvard Medical School.]

IN clinical literature scattered suggestions are to be found that emotional states may be the cause of more or less permanent glycosuria. Great grief and prolonged anxiety during a momentous crisis have been regarded as causes of individual instances of diabetes, and anger or fright has been followed by an increase in the sugar excreted by persons who already have the disease.[2] The onset of diabetes in a man directly after his wife was discovered in adultery is described by Naunyn, and this author also cites two cases in his own practice, — one started during the bombardment of Strassburg (1870), the other started a few days after a companion had shot himself.[3] In cases of mental disease, also, states of depression have been described accompanied by sugar in the urine. Schultze has reported that in these cases the amount of glycosuria is dependent on the degree of depression, and that the greatest excretion of sugar occurs in the fear-psychoses.[4] Raimann has reported that in both melancholia and mania the assimilation limit of sugar may be lowered.[5] And Arndt has observed glycosuria appearing and disappearing as alcoholic delirium appeared and disappeared in his patients.[6]

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[1] A preliminary announcement of the results of this investigation was made at the meeting of the American Philosophical Society, Philadelphia, April 22, 1911. See Proceedings, American Philosophical Society, 1911, l, p. 227; also Science, 1911, xxxiii, p. 907.

[2] See Kleen: On diabetes mellitus and glycosuria, Philadelphia, 1900, pp. 22, 37-39.

[3] Naunyn: Der Diabetes mellitus, Vienna, 1898, p. 72.

[4] Schultze: Verhandlungen der Gesellschaft deutscher Naturforscher und Ärzte, Cologne, 1908, ii, p. 358.

[5] Raimann: Zeitschrift für Heilkunde, 1902, xxiii, Abtheilung iii, pp. 14, 19.

[6] Arndt: Zeitschrift für Nervenheilkunde, 1897, x, p. 436.

Although clinical evidence thus indicates an emotional origin of some cases of diabetes and glycosuria, the intricacies of existence and the complications of disease in human beings throw some doubt on the value of that evidence. Both Naunyn and Hirschfeld, although mentioning instances of diabetes apparently due to an emotional experience, urge a skeptical attitude towards such statements.[7] It is desirable, therefore, that the question of an emotional glycosuria be tested under simpler and more controllable conditions. "Emotional glycosuria" in experimental animals has indeed been referred to by Waterman and Smit [8] and more recently by Henderson and Underhill.[9] Both these references, however, are based on the work of Böhm and Hoffmann, reported in 1878.[10] Böhm and Hoffmann found that cats, when bound to an operating board, tracheotomized (without anæsthesia), and in some instances catheterized by a suprapubic exposure of the urethra, had in about half an hour an abundance of sugar in the urine. In three determinations sugar in the blood proved slightly above normal so long as sugar was appearing in the urine, but returned to normal as the glycosuria disappeared. Since they were able to produce the phenomenon by simply binding animals to the holder, they called it "Fesselungsdiabetes."

As possible causes of this glycosuria in bound animals, they considered tracheotomizing, cooling, and pain. The first two they readily eliminated, and still they found sugar excreted. Pain they could not obviate, and since, without binding the animals, they caused glycosuria by merely stimulating the sciatic nerves, they concluded that painful confinement was itself a sufficient cause. Other factors, however, such as cooling and circulatory disturbances, probably cooperated with pain, they believed, to produce the result.

Inasmuch as Böhm and Hoffmann did not mention the emotional element in discussing their results, and inasmuch as they admitted that they could not obviate from their experimental procedure pain, which they themselves proved was effective in causing glycosuria, designating what they called "Fesselungsdiabetes" as "emotional glycosuria" is not justified.

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[7] NAUNYN: *Loc. cit.*, p. 73; HIRSCHFELD, Die Zuckerkrankheit, Leipzig, 1902, p. 45.

[8] WATERMAN and SMIT: Archiv für die gesammte Physiologie, 1908, cxxiv, p. 205.

[9] HENDERSON and UNDERHILL: This journal, 1911, xxviii, p. 276.

[10] BÖHM and HOFFMANN: Archiv für experimentelle Pathologie und Pharmakologie, 1878, viii, p. 295.

The discovery that during fright (or rage?) the adrenal secretion is increased,[11] and the fact that injection of epinephrin gives rise to glycosuria, suggested that glycosuria might be called forth by emotional excitement, and then that even without the painful element of Böhm and Hoffmann's experiments sugar might be found in the urine.

The first procedure, in testing this suggestion, was a repetition of Böhm and Hoffmann's experiments freed from the factor of pain. The animals (cats) were bound to a comfortable holder, which left the head unfastened, and which had been used hundreds of times in X-ray studies of digestion, with many different animals, without causing any signs of even so much as uneasiness.[12] Just as in observations on the movements of the alimentary canal, however, so here the animals reacted differently to the experience of being confined. Young males usually became quite frantic, and with eyes wide, pupils dilated, pulse accelerated, hairs of the tail more or less erect, they struggled, snarling and growling, to free themselves. Females, on the contrary, especially if elderly, were as a rule much more calm, and resignedly accepted the novel situation.

According to differences in reaction the animals were left in the holder for periods varying in length from thirty minutes to five hours (see Table I). In order to insure prompt urination, considerable quantities of water were given by stomach tube at the beginning of the experiment and in some cases again later. Arrangements were made for draining the urine promptly, when the animal was on the holder or when afterwards in a metal metabolism cage, into a glass receiver containing a few drops of chloroform. The diet in all cases consisted of customary raw meat and milk. In every instance the urine was proved free from sugar before the animal was used.

In the present series of observations twelve cats were used, and in every one a well-marked glycosuria was developed. The shortest periods of confinement to the holder which were effective were thirty and forty minutes; the longest we employed, five hours. The average time required to bring about a glycosuria was less than an hour and a half; the average in seven of the twelve cases (A, C, D, E, G, H, and K, Table I) was less than forty minutes. In all cases no sugar was found in the urine passed on the day after the excitement.

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[11] Cannon and de la Paz: This journal, 1911, xxviii, p. 64.

[12] Cannon: This journal, 1898, i, p. 363.

The promptness with which the glycosuria developed was directly related to the emotional state of the animal. Sugar was found early in animals which early showed signs of being frightened or in a rage, and much later in animals which took the experience more calmly (*cf.* E, F, and I in Table I with B, C, D, J, and K).

As cooling may result in hyperglycæmia, and consequent glycosuria, the rectal temperature was observed from time to time, and it was found to vary so slightly that, as far as these experiments might be concerned, it was a wholly negligible factor. In cat G the rectal temperature fell to 36° C. while the animal was bound and placed in a cold room (about 2° C.) for fifty minutes, but no sugar appeared in the urine.

Further evidence that the appearance of sugar in the urine may arise purely from emotional excitement was obtained from three cats (A, G, and H, Table I) which gave negative results when bound in the holder for varying periods up to four hours. It was noteworthy that these animals remained calm and passive in their confinement. When, however, they were placed separately in a small wire cage, and were barked at by an energetic little dog, that jumped at them and made signs of attack, the cats became much excited, they showed their teeth, humped their backs, and growled defiance. This sham fight was permitted to continue for a half-hour in each of the three cases. In each case the animal, which after four hours of bondage had exhibited no glycosuria, now had sugar in the urine. Pain, cooling, and bondage were not factors in these experiments, nor in the experiment in which cat E was barked at by the dog (see Table I). The animal was either frightened or enraged by the barking dog, and that excitement was attended by glycosuria.

The sugar excreted in the twenty-four hours which included the period of excitement was determined by the Bertrand method.[13] It ranged from 0.024 gm. to 1.93 gm., or from 8 mgm. to 0.62 gm. per kilo body weight, for the twenty-four-hour quantity.

Twenty-five samples of urine from the observed cats, twelve of the

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[13] See Abderhalden: Handbuch der biochemischen Arbeitsmethoden, Berlin, 1910, ii, p. 181.

TABLE I.

A SUMMARY OF THE CONDITIONS OF EMOTIONAL GLYCOSURIA AND THE AMOUNTS OF SUGAR EXCRETED.

Cat.	Sex.	Experience.	Remarks.	Urine 24 hrs.	Sugar excreted 24 hrs.	Sugar per kilo body wt.
				c.c.	gm.	gm.
A	Male	Bound 1½ hrs.	Calm	400	0.0	0.0
A	Male	Bound 2 hrs.	Calm	370	0.0	0.0
A	Male	Bound 4 hrs.	Calm	150	0.0	0.0
A	Male	Dog ½ hr.	Frightened	400	0.49	0.14
B	Male	Bound 1¾ hrs.	Excited	255	1.19	0.44
C	Male	Bound ¾ hr.	Excited	250	0.34	0.12
D	Female	Bound ¾ hr.	Excited	140	0.69	0.38
E	Female	Bound 1 hr.	Calm	220	0.0	0.0
E	Female	Bound 2 hrs.	Calm	185	0.0	0.0
E	Female	Bound 4 hrs.	Excited	358	1.93	0.62
E	Female	Dog ¾ hr.	Excited	90	1.10	0.35
F	Male	Bound 1½ hrs.	Calm	140	0.0	0.0
F	Male	Bound 1½ hrs.	Trembled	70	0.0	0.0
F	Male	Bound 4 hrs.	Fairly quiet	240	0.035	0.015
G	Male	Dog ½ hr.	Frightened	160	0.59	0.20
G	Male	Bound 2 hrs.	Calm	340	0.0	0.0
G	Male	Bound ⅝ hr., cold room	Rect. temp. 36	90	0.0	0.0
G	Male	Bound 4 hrs.	Calm	205	0.0	0.0
H	Male	Bound 2 hrs.	Slightly excited	160	0.0	0.0
H	Male	Bound 4 hrs.	Calm	115	0.0	0.0
H	Male	Dog ½ hr.	Frightened	65	0.024	0.008
I	Female	Bound 3 hrs.	Calm	155	0.0	0.0
I	Female	Bound 5 hrs.	Cried; struggled	130	0.55	0.25
J	Female	Bound 1¼ hrs.	Cried; struggled	215	0.78	0.31
K	Female	Bound 1 hr.	Raging	95	+	...
L	Male	Bound 1 hr.	Calm	...	0.0	0.0
L	Male	Bound 1¼ hrs.	Raging	...	+	...

samples containing sugar, were tested for acetone by Legal's sodium nitro-prusside method, but were found negative.

To what extent the observations here recorded on cats will prove true for other animals used for experimentation remains to be investigated. Fischer has reported that he bound rabbits to a holder for periods lasting from twenty-four to thirty-six hours without causing sugar to appear in the urine.[14] Rabbits, however, often seem to be stupefied rather than excited by this confinement. Gib has given an account of a bitch that became much agitated when shut up, and after such enforced seclusion, but never otherwise, excreted small quantities of glucose in the urine.[15] Further observations on the urine of experimental animals in states of excitement are desirable.

Since splanchnic stimulation produces glycosuria,[16] and since major emotions, such as rage and fright, are attended by nervous discharges along splanchnic pathways, glycosuria as an accompaniment of emotional excitement might be expected to occur. To what extent the adrenal glands which, as already mentioned, are stimulated to increased secretion by excitement, might play a part in this process, is still in dispute. Adrenalectomy or denervation of the adrenals, according to some observers,[17] prevents glycosuria after puncture of the fourth ventricle, and also after stimulation of the splanchnic nerves.[18] On the other hand Wertheimer and Battez state that removal of the adrenal capsules does not abolish the effects of piqure in the cat.[19] It was questionable, therefore, whether removal of the adrenal glands would affect emotional glycosuria.

Evidence on this point was secured in observations on three animals that were decapsulated aseptically under ether. The animals selected (C, K, and L, Table I) had all become quickly excited on being bound to the holder, and had manifested glycosuria in about an hour of confinement. After opening the abdomen the adrenal veins were first tied, and then the capsules freed from their attachments and removed as quickly and with as little handling as possible. In cat K the entire operation was finished in twenty minutes. In cats K and L a small catheter was introduced into the urethra through an incision, so that the bladder could be emptied at any time.

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[14] FISCHER: Archiv für die gesammte Physiologie, 1905, cix, p. 13.

[15] Cited by KLEEN: *Loc. cit.*, p. 37.

[16] See MACLEOD: This journal, 1907, xix, p. 405, also for other references to literature.

[17] See MEYER: Comptes rendus de la Société de Biologie, 1906, lviii, p. 1123; NISHI, Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, p. 416.

[18] GAUTRELET and THOMAS: Comptes rendus de la Société de Biologie, 1909, lxvii, p. 233; and MACLEOD: Proceedings of the Society for Experimental Biology and Medicine, 1911, viii, p. 110 (true for left capsule and left splanchnic).

[19] WERTHEIMER and BATTEZ: Archives internationales de physiologie, 1910, ix, p. 392.

In all three cases urine was obtained soon after the operation that was free from sugar. Although the decapsulated animals manifested a general lessening of muscular tone, they still displayed much of their former rage or excitement when bound. Indeed cat L was more excited after decapsulation than before. That the animals might not be excessively cooled they were kept warm with coverings or an electric heating pad. Although they were now bound for periods from two to three times as long as the periods required formerly to cause glycosuria, no trace of sugar was found in the urine in any instance. The evidence thus secured tends, therefore, to support the view that the adrenal glands perform an important rôle in the glycosuria resulting from splanchnic stimulation.

The question arises at this point as to the interpretation of the results reported by Böhm and Hoffmann in their paper on "Fesselungsdiabetes." Possibly the emotional element was in part accountable for the glycosuria which they observed, but the pain which they produced, as they themselves recognized, would reasonably explain it. Strong stimulation of sensory fibres will cause discharges along the splanchnic nerves, and incidentally will bring about an increased secretion of the adrenal glands.[20] In glycosuria resulting from painful stimulation, therefore, as well as in emotional glycosuria, the adrenal glands may be essential factors.

There is little doubt that the glycosuria following emotional excitement, like that resulting from pain, is dependent on a state of hyperglycæmia. Conceivably such hyperglycæmia may be useful to the organism. MacDougall, in associating instincts and emotions, has put together "flight instinct" and "fear emotion," and "pugnacity instinct" and "anger emotion." [21] Both the fear emotion and the anger emotion are, in wild life, likely to be followed by activity requiring contraction of great muscular masses in supreme and prolonged struggle. A mobilization of sugar in the blood under these circumstances might be of signal service to the laboring muscles

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[20] CANNON and HOSKINS: This journal, 1911, xxix, p. 278.

[21] MACDOUGALL: Introduction to social psychology, London, 1908, pp. 49, 59.

SUMMARY.

When cats are excited for even so short a time as one half-hour, either by being bound in a comfortable holder or by being caged and barked at by an active dog, they exhibit glycosuria. Neither pain, cooling, nor being bound, therefore, need be a factor in the result — the fright or the rage of the animal is the essential element.

The amount of sugar per kilo of body-weight, found in the urine during the twenty-four hours which included the excitement, averaged 0.26 gm. with a maximum of 0.62 gm.

Animals which had glycosuria when bound for about an hour failed to have it after careful adrenalectomy, although bound between two and three times as long as before, and still manifesting the excitement which they had manifested previous to the operation.

On the reasonable assumption that the emotional glycosuria here described is due to hyperglycæmia, a possible utility of the sugar mobilization in the blood is suggested.

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AN ATTEMPT TOWARD THE PHYSICAL CHEMISTRY OF THE PRODUCTION OF ONE-EYED MONSTROSITIES.

By J. F. McCLENDON.

[*From the Embryological Laboratory of Cornell University Medical College, New York City, and the U. S. Bureau of Fisheries Laboratory, Woods Hole, Mass.*]

EXPERIMENTAL teratology is one of the oldest branches of the physiology of development. However, the majority of the experiments have been performed on difficult material, such as the eggs of frogs, birds, and mammals, and with uncertain results. Stockard[1] observed that cyclopia, or the numerical defect in the eyes, may be easily produced in embryos of the marine fish, Fundulus heteroclitus. He found the defects to arise, apparently, from the retardation in the growth of the brain region lying between the Anlagen of the two optic vesicles. Probably this region is more sensitive than any other to toxic substances.

No one has recorded cyclopia in Fundulus embryos kept under normal conditions. A number of monstrosities develop from the eggs of fish, especially those, such as the trout, which require a very high oxygen tension for their development. When the temperature rises, and consequently the oxygen tension falls, many abnormal forms appear. However, I know of no observations, except my own, on the repeated natural occurrence of cyclopia in fish. During two successive seasons I found about one tenth of one per cent of the smelt embryos, at Cold Spring Harbor, Long Island, developed in streams, or in hatching jars of the New York State Fish Hatchery, to be cyclopic. Stockard observed abnormalities in Fundulus embryos developed in the fresh water at this place.

I have attempted to find the cause of these abnormalities, but have not completed this work. In fact, only one clue has been found, and that is the CO_2 content of the water. The fresh water at Cold Spring Harbor comes from deep artesian wells and springs, and is heavily charged with carbonic acid. Dr. R. A. Gortner found more than ten times as much CO_2 in some samples of water that had just emerged as was found in pond water. Ordinary distillation did not change the CO_2 content. It seems probable that the carbonic acid is the cause of cyclopia in these smelt embryos, but I have found no proof of this assumption. The eggs are deposited naturally in large masses, with spaces between the eggs. Those on the surface of the masses develop faster, due probably to larger amount of oxygen or light, or smaller amount of CO_2. But in the hatching jars the eggs are separated, and all are exposed to the same conditions.

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[1] Stockard: Journal of experimental zoölogy, 1907, iv, p. 165; 1909, vi, p. 286; American journal of anatomy, 1910, x, p. 369.

I produced cyclopia in Fundulus embryos with a number of salts and with volatile anæsthetics. The solutions might be very hypertonic or very hypotonic. On the one hand, distilled water containing only traces of dissolved substances or substances to which the egg is freely permeable, were effective, while, on the other hand, 3.4 volumes of sea water concentrated to one volume were likewise so. It seems, therefore, that osmotic pressure is not primarily the cause of cyclopia.

However, the eggs decreased in volume when placed in theoretically isomotic solutions producing cyclopia, tending to show that these solutions increased the permeability of the egg to the substances producing the internal osmotic pressure, or turgor. We need only to suppose that the cells between the eye Anlagen are more easily affected than other cells of the embryo, to explain the action of the solutions. The decrease in turgor would retard growth in the same way as a hypertonic solution prevents development.

It is well known that great increase in permeability means death, and that death is always accompanied by increased permeability. In my experiments the concentration of salts or anæsthetics causing cyclopia was always just slightly below the lethal dose (Table I). The eggs were placed in the solutions at the two-cell stage, and usually remained in these solutions until the eyes appeared. Those apparently cyclopic were removed to sea water and observed more minutely at a later date, then finally made into histological preparations.

In one case cyclopia was observed in distilled water containing minute traces of heavy metals,[2] and it will be observed from Table I that hypotonic solutions of NaCl and LiCl produced the same result as the traces of metal. The addition to sea water of the chlorides of Na, Li, or Mg, thus raising the osmotic pressure, produced cyclopia.

TABLE I.

Substance.	Dist. water solutions.		Sea water solutions.	
	Lethal dose.	Cyclopic dose.	Lethal dose.	Cyclopic dose.
KCl	.....	...	.8–.9 mol.	.....
NaCl	.4–.5 mol.	.3–.36 mol.	1.2–1.6 "	1.04–1.36 mol.
LiCl	.2–.25 "	.2 "	.66 "	.33–.66 "
$MgCl_2$	.4–.5 "	...	.4–.5 "	.3–.38 "
$CaCl_2$	.2–.3 "	...	.2–.3 "	.....
NaOH	.006–.01 mol.	.006 "	>.01 "	.....
HCl	[1] .00015-.0002 mol.	...	.0025 "	.....
Cane sugar	1. molecular	...	>.5 molecular	.....
Dextrose	.....	...	1.–2. "	.....
Methyl alcohol	.....	...	<1.8 vol. %	.....
Ethyl "	3.15–4.75 vol. %	3 vol. %	3.5–4.75 vol. %	2–3.66 vol. %
Amyl "	.....	...	.2 "	.1–.15 "
Acetone	.....	...	3.2 "	1.6–2.4 "
Phenol	.....	...	<1 "	.....
Ether	.....	...	1/6 saturated	1/8 saturated

[1] At about the same concentration KAHLENBERG and TRUE, Journal of the American Medical Association, July 18, 1896, p. 138, found the growth of certain plants to cease and showed that this was due to H^+ ions.

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[2] The traces of metal came from the still, and the experiment was designed to test the effect of water distilled in metal. The distilled water used in all other experiments was re-distilled in a fused quartz condensing tube, and there was no cork, rubber, or other organic material in the apparatus.

Stockard[3] obtained one cyclops monster by adding $MnCl_2$ to sea water.

It is interesting to note that merely the alteration of the relative concentration of the salts already present in sea water produces cyclopia. In order that these salts should act on the region between the eye Anlagen after the neural tube has formed, they must first penetrate the embryo. According to Overton and others, living cells are relatively impermeable to electrolytes. Therefore I determined the permeability of the Fundulus egg under the conditions of the experiments in which cyclopia was produced. The eggs were impermeable to Cl ions, but permeable to the kations. By a kationic exchange, the salt content of the interior could be qualitatively but not quantitatively altered (see Appendix II).

Anæsthetics belong to the class of substances which freely penetrate living cells. They also change the permeability of cells to other substances. Czapek[4] observed that many anæsthetics increase the permeability of plant cells to the contained tannin, in "iso-capillary" concentrations. By iso-capillary he designates solutions which have the same surface tension. The anæsthetics which behave in this way are the so-called indifferent anæsthetics, *i. e.*, substances which alter the cells physically but not, or to a small extent, chemically, and whose effects are wholly reversible unless the concentration producing narcosis is far exceeded.

In order to determine whether this rule applied to cyclopia, I made iso-capillary solutions of various anæsthetics and observed their relative effects. Some difficulties were met in determining the surface tension of the solutions. According to I. Traube, in homologous series of anæsthetics, a molecule of each member is three times more powerful in lowering the surface tension than a molecule of the preceding member. However, Traube's rule appears to be only approximate, and difficulties are met in determining the molecular concentration of the solutions. Therefore I measured the surface tension directly with Traube's stalagmometer, making corrections for specific gravity. The apparatus used by Czapek was tried but given up.

Since the surface tension was measured directly, there was no necessity of having the substances absolutely pure, although the character of impurities would be significant. I used absolute ethyl alcohol and pure ether, acetone and phenol, but the other substances were not pure.

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[3] STOCKARD: Journal of experimental zoölogy, 1907, iv, p. 187.

[4] CZAPEK: Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen, Jena, G. Fisher, 1911.

Large glass bottles were partially filled with the solutions, a relatively small number of eggs in the two-cell stage added, and the bottles tightly corked and set aside for forty-eight hours; the eggs were then transferred to sea water. In the tables, *Cy.* signifies the production of true cyclopia (1 median eye), *Monop.* indicates monopthalmia asymmetrica (one lateral eye normal, the other vestigial or absent).

TABLE II.

Iso-capillary solutions.	6 per cent methyl alcohol.	3.36 per cent ethyl.	.2 per cent amyl.	3.2 per cent acetone.	1/6 saturated ether.	1 per cent phenol.
Undiluted . .	All dead	Nearly all dead, cy.	All dead	All dead	All dead	All dead.
Diluted to three fourths.	" "	Cy.	Monop.	Cy	Cy.	" "
Diluted to one half	" "	Cy.	Cy.	Cy.	. . .	" "
Diluted to three tenths.	" "	. . .	. . .	. .	. . .	" "

Table II shows that cyclopia-producing solutions of ethyl and amyl alcohol, ether[5] and acetone, are iso-capillary, also the lethal doses are iso-capillary. Ethyl and amyl alcohol and ether have been classed as indifferent anæsthetics. Acetone may be considered as almost indifferent chemically, at least to many cells.

All of the solutions of methyl alcohol or phenol that were used killed the eggs. These substances probably act chemically on the eggs. Czapek found methyl alcohol to fall into the class of indifferent anæsthetics as regards plant cells. It may be objected that the toxic action of my sample was due to impurities, but evidently a contamination with amyl alcohol and acetone could not be responsible for the effect, since these substances behave as indifferent anæsthetics, and their action is additative.

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[5] My results with ether are not as exact as with the other substances. The high vapor tension of ether prevented exact measurements of surface tension of its solution. If the drops were in contact with circulating air, the concentration of ether in the surface of the drop was lowered. If the drops fell into an enclosed space, the ether vapor in the air altered the surface tension of the drop.

Feré[6] found the teratogenic power of alcohols injected into hen's eggs, measured in volumes per cent, to fall in the following series, ethyl<prophyl, butyl<amyl. Since, according to Traube, each member of this series lowers the surface tension of water three times more powerfully than the one preceding, we may conclude that they were equally effective in "iso-capillary" concentrations. However, the data of Feré are too inadequate for any final conclusions.

It is interesting to note that Child[7] produced cyclopia in flat-worms, Planaria, with anæsthetics.

Appendix I (Tables).

KCl in sea water:

Molecular concentration	.3 to .8	.9
Result	50 per cent dead.	All dead.

Pure NaCl:

Molecular concentration	.2	.3	.35	.4
Result	All hatched.	None hatched, monop.	Monop.[1]	Cy.[2]

Molecular concentration	.4	.5–1
Result	Nearly all dead.	All dead.

NaCl in sea water:

Molecular concentration	.9	1.	1.04	1.12
Result	All normal.	59 per cent dead.	Cy.	Cy.

Molecular concentration	1.2	1.28	1.36	1.6–2.0
Result	60 per cent dead, cy.	Monop.	Cy., monop.	All dead.

Pure LiCl:

Molecular concentration	.2	.25	.3–.4
Result	Cy.	Nearly all dead.	All dead.

LiCl in sea water:

Molecular concentration	.33	.4	.5	.6	.66
Result	All normal.	Monop.	Monop.	Monop.	Monop.

[1] Monop. = monopthalmia asymmetrica. [2] Cy. = cyclopia.

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[6] Feré: Cinquantenaire de la Société de Biologie, 1899, p. 360. Reviewed in Ballentyne: Antenatal pathology and hygiene, Edinburgh, Green & Co., 1904, ii, p. 210.

[7] Child: Biological bulletin, 1909, xvi, p. 277; 1911, xx, p. 309.

Pure $MgCl_2$:

Molecular concentration	.3	.4	.5
Result	75 per cent dead.	95 per cent dead.	All dead.

$MgCl_2$ in sea water:

Molecular concentration	2.8	3	.32	.33
Result	53 per cent dead.	57 per cent dead, monop.	63 p. c. dead, monop, cy.	Cy.
Molecular concentration	.34	.36	.38	.4
Result	Monop, cy.	83 per cent dead, cy.	88 per cent dead, monop.	97 per cent dead.

$CaCl_2$ in sea water:

Molecular concentration	.3
Result	All dead.

Pure $CaCl_2$:

Molecular concentration	.2	.3
Result	30 per cent dead.	All dead.

Volumes of sea water concentrated to one volume:

1.9	2.2	2.5	2.65	2.8	2.95	4.
50 per cent dead.	42 per cent dead.	28 per cent dead, monop.	Cy.	62 per cent dead, monop., cy.	Monop.	All dead.

APPENDIX II (PERMEABILITY OF THE EGG).

It was thought desirable to know something of the composition, especially the salt content, of the egg, but the analysis was not completed. After remaining one hour in sea water, 44.318 gm. of unfertilized eggs were wiped dry with filter paper. After desiccation to constant weight, the dry substance was found to be 20 per cent of the total. The dry substance contained:

Alcohol-ether extract	12.2	per cent.
Insoluble ash	5.47	" "
Soluble ash	3.18	" "

The chlorides in the soluble ash = 4.82 c.c. of a normal solution. Therefore the chloride content of the water in the eggs (35.576 c.c.) was about .135 normal, or only .27 as much as in the sea water, which contained from .49 to .51 normal.

An estimate of the magnesium content may be made from the following, although the sample was too small for accurate results. Ten gm. eggs contained 2 gm. dry substance. The ash analyzed for Mg by the method of W. Gibbs gave .0336 gm. magnesium pyrophosphate. From this the magnesium content in the 8 c.c. of water in the eggs may be calculated as .03 molecular, or one half as much as in sea water.

From these preliminary ash analyses we might infer that the eggs, or certain regions within them, are impermeable to Cl and Mg ions and $MgCl_2$. However, it appeared to me that this might be determined by dialysis, especially since these eggs will live in distilled water.

In the following experiments the water was re-distilled in quartz. The eggs were fertilized and washed in many changes of water for about two hours. They were then placed in a graduate, and water or salt solution added to make 100 or 200 c.c., and soaked for a certain length of time. Three fourths of the total volume was decanted, evaporated, charred, and analyzed. The eggs were placed in a second volume of the solution and the process repeated.

Vol. of eggs.	Solution.	Hour.	T. Cl.[1]	Hour.	T. Cl.	Hour.	T. Cl.	Hour.	T. Cl.	Hour.	T. Cl.
12	H_2O	2d	None	16th	None	41	None	..	..	..	..
12	$M/4MgSO_4$	2d	None	"	"	"	"	..	..	..	..
12	H_2O	2d	None	"	"	"	"	68	None	113	None
12	Nitrates	2d	None	"	"	"	"	"	"	"	"

[1] T. Cl. = chlorine by titration.

The above table shows the egg to be impermeable to chlorine ions. The nitrates were of the same kations as are contained in sea water.

The above table shows a kationic exchange, Na in the medium being exchanged for Mg in the eggs. No Mg came out of the eggs in distilled water because the Mg^+ ions were held back by the electric charge of the Cl^- ions in the egg. The ash analysis given above suggests that the egg is normally impermeable to Mg ions, and that the pure NaCl solution, or Na+K, may increase its permeability to Mg.

In this connection it must be emphasized that it is the plasma membrane or surface of the protoplasm of the egg that shows this impermeability, and not the relatively firm egg shell, or chorion. I have demonstrated the permeability of the egg shell, or chorion, of fish eggs to several salts by microchemical methods. Loeb[8] suggests that change in permeability of the egg to salts may be due to the fact that the micropyle is at times open and at others closed. Since the micropyle is a hole in the egg shell, its stoppage cannot prevent the diffusion of salts, as the shell is permeable to them.

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[8] Loeb: Biochemische Zeitschrift, 1911, xxxvi, 275.

Vol. of eggs.	Solution.	Hour.	Mg. precip. in milligrams.	Hour.	Mg. precip.	Hour.	Mg. precip.
17	H_2O	19th	None	33	None	129	None
17	N/10 NaCl	"	.6[1]	"	Dense cloud	"	Dense cloud
10	H_2O	29	None	..	...	..	...
10	N/10 NaCl	"	Dense cloud	..	...	..	...
20	N/10 NaCl	90	None	..	...	..	...
20	" "	"	Dense cloud	..	...	..	...

[1] If we can rely on these small quantities, this represents .005 of the total milligrams in the eggs.

APPENDIX III ("LITHIUM EMBRYOS").

Lithium produces a large per cent of embryos with enlarged segmentation cavities, which were observed and described by Stockard,[9] who called them "lithium embryos." I have confirmed this observation, and found similar embryos in pure solutions of .38 to .5 molecular NaCl, and in sea water solutions of ether, acetone, molecular dextrose, and .3 mol. $CaCl_2$, but in these latter the per cent of the "lithium embryos" is less than in solutions of LiCl. These embryos were studied alive, then prepared histologically and studied as serial sections. Those produced by lithium did not differ from the others.

Apparently the fluid from the segmentation cavity migrates forward at a later period and comes to lie in front of the embryo, thus producing a vesicle in the pericardial region. However it is possible that these two collections of fluid have different origins.

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[9] STOCKARD: Journal of experimental zoölogy, 1906, iii, p. 99.

DYNAMICS OF CELL DIVISION. — III. ARTIFICIAL PARTHENOGENESIS IN VERTEBRATES.

By J. F. McCLENDON.

[*From the Embryological Laboratory of Cornell University Medical College, New York City; and the Station for Experimental Evolution of the Carnegie Institution of Washington, at Cold Spring Harbor, Long Island.*]

I. By Mechanical Stimulation.

EGGS of the wood frog, Rana sylvatica, and the tree frog, Hyla pickeringii, were caused to segment by momentary pressure. Cutting the mass of eggs from the uterus to pieces with scissors caused a few eggs to die and a few others to segment. Compression was a very unreliable method for causing segmentation, and much more uniform results were obtained by pricking with a fine needle.[1]

The females were washed with alcohol followed by a strong stream of water. The eggs were carefully taken from the uterus so as to avoid pressure and placed in shallow dishes. In some experiments the eggs were pricked immediately and then covered with water. In others the eggs were first covered with water and the jelly allowed to swell slightly, though the eggs remained adherent to the bottom and were thus held in position. The glass dish was placed under a Zeiss binocular microscope and the eggs lightly pricked with the finest sewing needle.

The extent of the puncture had to be regulated with great care in order to obtain good results. It seemed necessary merely to touch the surface of the egg in order to start development, although a puncture that did not result in a large extra-ovate was permissible. In my operations on thousands of eggs, some failed to be reached by the needle, whereas some others were pricked too deeply and died. The jelly offered considerable resistance, and a sudden stab with the needle seemed less injurious than a slow motion.

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[1] Bataillon: Comptes rendus de la Société de Biologie, 1911, lxx, p. 562, had previously caused European frog's eggs to segment by pricking with platinum or glass needles. He claims to have developed a few to the metamorphosis. He interprets Guyer's results on injecting lymph into frog's eggs as parthenogenesis due to pricking, but thinks the serum entering the wound may be essential.

Eggs which were pricked just sufficiently, rotated in the normal manner and segmented. The first cleavage was regular in a small per cent, and showed varying degrees of irregularity in the remainder. No regular later cleavages were observed, so that it is impossible to state how many cytoplasmic cleavages occurred, though divisions of the nuclei continued for a few days, not, however, at the normal rate.

In some cases the first cleavage furrow passed through the point of puncture, but since it did not do so in all cases, further study would be necessary to analyze the localization factors.

A control was always kept, care being taken not to subject any of the eggs to pressure. None of these eggs segmented, but it was noted in some cases that they finally became wrinkled and apparently decreased in volume. According to Biataszewicz,[2] the unfertilized frog's egg swells continuously. The water which I used came from an artesian well, and contained over ten times as much CO_2 as was found in pond water,[3] and it is possible that the CO_2 injured the eggs. In harmony with this view is the fact that there was a large mortality among fertilized eggs developing in it.

II. By Electrical Stimulation.

Eggs of the wood frog, Rana sylvatica, the tree frogs Hyla pickeringii and Hyla versicolor, and the toad, Bufo lentiginosus, were caused to segment by electric stimulation. The character of segmentation was similar to that in pricked eggs. A high per cent could be caused to segment. The segmentation rate seemed to be about normal, for instance the first cleavage in Hyla pickeringii occurred from two to three hours after electric stimulation, at 21° C. But in order to avoid the slightest possibility of contamination with sperm, no males or fertilized eggs were allowed in the laboratory during the experiments, and the rate of development was not directly compared with the normal.

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[2] Biataszewicz: Bulletin de l'Académie des Sciences de Cracovie, 1908, p. 783.

[3] From analysis by R. A. Gortner.

The females were washed with alcohol followed by a strong stream of water. The eggs were carefully taken from the uterus, without pressure, placed in glass dishes and covered with water. An alternating current of 110 volts and 60 cycles was passed through the water from platinum or carbon electrodes held from 2.5 to 15 cm. apart. The use of an alternating current prevented appreciable polarization and chemical changes at the electrodes, but in order to avoid the possibility of the slightest chemical influence, the water was poured off of the eggs immediately after removal of the electrodes and fresh water added. Eggs very close to the electrodes were necessarily more affected by the current than those farther removed, but in comparing experiments I have regarded only those eggs midway between the electrodes. The position of the electrodes was marked, and the eggs remained adhering to the glass in their original positions.

An instantaneous exposure to the current was sufficient and prolonged exposure injurious, although the resistance of the eggs to injury varied with the species. Having the electrodes 5 cm. apart, the injurious effect was seen in Hyla pickeringii eggs after five seconds, but in toad's eggs only after twenty to one hundred and twenty seconds' exposure.

The majority of the experiments were performed in artesian water. The fact that the use of water re-distilled from barium hydrate gave as good results, indicates that the CO_2 of the artesian water was not essential to parthenogenesis. The addition of 1/50, 1/20, 1/10, or even 1/5 vols. of sea water did not prevent cleavage. Also the following pure salts and combinations were permissible: $NaCl$, $CaCl_2$, $MgCl_2$, Na_2CO_3, $CaOH$, chlorides of Na & Ca, Ca & Mg, Na & Ca & Mg.

A large number of eggs of each species were removed at various periods after stimulation and prepared histologically in serial sections. The following account of the internal changes in eggs of the wood frog applies to all except for details as to size, amount of pigment, etc.

The female pro-nucleus, in passing from the surface at the animal pole, toward (but not reaching) the centre of the egg, leaves a pigment track similar to that left by the sperm nucleus in fertilized eggs.[4] I have no doubt that this pigment track is present in normal development, but has not been observed because the first cleavage furrow cuts through it longitudinally, as it is being formed, and obliterates it. In parthenogenetic eggs the furrow is usually not through the axis of symmetry, in which the pigment track lies, and therefore usually does not obliterate it.

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[4] In Hyla pickeringii, where there is little pigment, the track is hardly noticeable.

Although the first cleavage is not usually through the axis of symmetry, it is often near this axis, and therefore very nearly regular. Sometimes a large cell is cut off from a small one. In the majority of cases, however, two and sometimes more furrows appear simultaneously. This is not due to a corresponding increase in number of nuclei, as sections show but one nucleus, which is the undivided female pro-nucleus. The metaphase of the first nuclear division is reached long after the first cleavage furrow begins to cut through the egg.

The first cleavage usually does not completely cut through the vegetative pole. Later cleavage furrows sometimes appear, but are likewise never completed.

Cleavage of the cytoplasm is brought to a standstill, and the nuclear changes continue. The nucleus divides repeatedly, filling the egg with daughter nuclei. Finally the egg becomes vacuolated and is dead within a few days.

In a preliminary note[5] I have mentioned certain probabilities in regard to the theoretical significance of these results. However, since more data are desirable, I will not continue the discussion here, but hope to do so at a later date.

[5] McCLENDON: Science, 1911, N. S. xxxiii, p. 629.

THE INCREASED PERMEABILITY OF STRIATED MUSCLE TO IONS DURING CONTRACTION.

BY J. F. McCLENDON.

[*From the Embryological Laboratory of Cornell University Medical College, New York City.*]

ACCORDING to the membrane theory of Bernstein,[1] the muscle fibre is surrounded by a plasma membrane or surface film, allowing easier exit to one or more classes of kations than to the corresponding anions. The kations of some electrolyte which is more concentrated within than without come through the surface film and give the surface a positive electric charge. Destruction or alteration of this film or membrane causes a negative variation (the affective surface being less positive than the normal surface).

It has been suggested that potassium ions, which are more concentrated within the muscle than in the blood plasma, give the muscle the positive charge. However, one should then expect a reversal of the electric effects by placing muscle in an isotonic solution of potassium salts. This was shown by Höber[2] and Overton[3] not to occur. R. Lillie[4] suggests that lactic and carbonic acids are the electrolytes in question, and the H ions give the muscle surface the positive charge. The difficulty with this view lies in the fact that muscle, as shown by Overton, is in general permeable to substances soluble in many oils. It seems, therefore, that this question is not settled.

If the negative variation or "action current" of muscle is due to increased permeability to any ions, we should expect increased electric conductivity on contraction. It has long been known that the electric conductivity of muscle increases at death. Du Bois Reymond[5] explained the electric resistance of living muscle by the presence of membranes, which become altered (permeable) at death. He showed that muscle becomes polarized on the passage of an electric current. It seems to me that Kodis[6] and Galaeotti[7] take a step backward in attributing the increased conductivity of dead muscle to the liberation of ions. Galaeotti tried to support his view by determinations of the freezing points of living and dead muscle, but found, on the contrary, that the change in electric conductivity did not correspond to the change in the osmotic pressure.

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[1] BERNSTEIN: Archiv für die gesammte Physiologie, 1902, xcii, p. 521.

[2] HÖBER: Archiv für die gesammte Physiologie, 1905, cvi, p. 607.

[3] OVERTON: Sitzungsberichte der physikalisch-medizinische Gesellschaft, Würzburg, 1905, p. 2.

[4] LILLIE: This journal, 1909, xxiv, p. 14; 1911, xxviii, p. 197.

I have not found in the literature a clear statement that the electric conductivity of muscle has ever been measured, actually, during contraction. The contraction period (about one fifth of a second for frog's muscle) is too short for an accurate measurement of conductivity to be made. Therefore I decided to measure the conductivity during tetanus.

EXPERIMENTS.

Platinum electrodes similar to those designed by Galaeotti were used. These were "platinized" with a solution of platinic chloride containing a trace of lead acetate. Since the muscle was in contact with the electrodes, there was danger of rubbing off some of the platinum black. I found that the black adhered more strongly if the electrodes were previously roughened by coating with platinum black and then heating in a flame.

The method of Kohlrausch was used to measure the conductivity. The smallest sized induction coil made for such experiments was fitted with a rheostat in the primary, and only just enough current was used as is required to work the interrupter. A second rheostat was inserted in the secondary, and the current could be reduced until it was not felt when passed through my tongue.

The muscles of the frog's thigh were used. They were placed between, and with the fibres parallel to, the electrodes, and the latter pressed together until the muscle bulged out on all sides. The electrodes were then fixed rigidly in position and the preparation placed in a moist, constant temperature chamber.

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[5] DU BOIS REYMOND: Untersuchungen über tierische Electricität, 1849.

[6] KODIS: This journal, 1901, v, p. 267.

[7] GALAEOTTI: Zeitschrift für Biologie, 1902, n. F. xxv, p. 289, and 1903, xxvii, p. 65.

The conductivity of the muscle was measured with too little current to cause contraction. Then, by cutting out resistance in the secondary circuit, enough current was passed to throw the muscle into tetanus and a second reading made. In all cases the conductivity was greater during contraction.

It might be objected that the heating effect of this current would change the conductivity, but control experiments on liver tissue and the tissues of certain plants, in which no change in conductivity occurred, showed this not to be the case.

Since the muscle at all times entirely filled the space between the electrodes and extended out on all sides, a change in the form of the entire muscle would not appreciably alter the conductivity. However, it is possible that the change in form of the muscle fibres might slightly alter the conductivity, but it is improbable that it would account for the large differences observed.

If the change in conductivity were due to metabolic activity in the muscle, we would expect different results depending on whether the muscle was measured first in the stimulated or unstimulated condition, but no such difference was found. If the change in conductivity is due to chemical change, the latter must be completely and instantaneously reversible.

Experiments were made both in the spring and the autumn, and a long series of measurements were made on each muscle. The increase in conductivity was greatest in fresh preparations and decreased as the muscle became fatigued, varying from 28 to 6 per cent.

Discussion of Results.

The increased conductivity of muscle during contraction may be interpreted as demonstrating the increase in permeability of some structures within the muscle to anions (since the muscle appears already permeable to certain kations). According to the membrane theory, this causes a reduction of the electrical polarization of these structures, thus causing increased surface tension and contraction.

In order that the contraction be due to increased surface tension of any structures, the latter must during relaxation be elongate in the axis of the muscle. We have such a structure in the anisotropic segment of an ultimate fibril. It is interesting to note that Duesberg[8] finds these segments to arise from fat or lipoid-containing bodies known as chondriosomes, and the presence of fat would account for the high surface tension between them and the sarcoplasm that is necessary for contraction.

Bernstein calculated the size of the structures that would be compatible with the force of contraction, and concluded that it must be smaller than any structures seen in histological preparations of muscle. He therefore postulated hypothetical ellipsoids as the elements in question.

It is possible that surface tension changes are aided by osmotic pressure,[9] since the movements of plants are due to osmotic changes following changes in permeability. However, the small size of the muscle elements makes it impossible to apply botanical methods to it.

[8] DUESBERG: Archiv für Zellforschung, 1910, iv, p. 602.

[9] *Cf.* MEIGS: This journal, 1910, xxvi, p. 191.

ON THE INFLUENCE OF INTRAVENOUS INJECTIONS OF AN EXTRACT OF THE PANCREAS ON EXPERIMENTAL PANCREATIC DIABETES.

By ERNEST LYMAN SCOTT.

[*From the Hull Laboratories of Physiology and Biochemistry.*]

IN spite of the many attempts to lower the sugar output in pancreatic diabetes by the administration of extracts of the pancreas, no one seems to have obtained consistent, positive results, or, as Hédon[1] has recently pointed out, the positive results are capable of several interpretations. A good review of the subject will be found in the papers of Leschke[2] and Pflüger.[3] Leschke showed that the external digestive enzymes of the pancreas have a fatal effect upon the experimental animal; but, in his attempts to destroy these enzymes by heat he evidently destroyed the internal secretion as well, if in fact it was present in active form in his extracts. That these external enzymes may destroy the internal secretion also is hinted at by Cohnheim[4] and his followers in their method of extraction. Another point that seems not to have been sufficiently considered is the possibility that the internal secretion may be readily destroyed by oxidation during the preparation. This seems probable in the light of our experience with adrenalin. That the secretion is at least very unstable is indicated by Drennan's work.[5] This investigator injected sterile blood from normal dogs into depancreatized dogs and found in every case a diminution of the sugar output in the urine, provided the blood was fresh, but if the blood had stood for a few hours after being drawn, no results followed. It seemed worth while, then, to see if the sugar output could be lowered by an extract in which precautions were taken to prevent (*a*) the effect of the digestive enzymes on the internal secretion as well as on the subject, and (*b*) to prevent the oxidation of the internal secretion while being prepared from the gland.

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[1] HÉDON: Comptes rendus de la Société de Biologie, 1911, lxxi, p. 124.

[2] LESCHKE: Archiv für Physiologie, 1910, p. 401.

[3] PFLÜGER: Archiv für die gesammte Physiologie, 1907, cxviii, p. 263.

[4] COHNHEIM: Zeitschrift für physiologische Chemie, 1904, xlii, p. 401.

[5] DRENNAN: This journal, 1911, xxvii, p. 396.

It was hoped that the presence of the digestive enzymes could be eliminated by the atrophy of the gland which follows complete ligation of the ducts; but after several attempts in the dog which proved futile so far as complete atrophy was concerned, this method was abandoned as impractical. In subsequent work these enzymes were rendered inactive at once by a high percentage of alcohol and were later killed by long-continued contact with strong alcohol.

Methods of preparing the extracts. — Two methods of extraction were attempted: (*a*) by alcohol, and (*b*) by water rendered slightly acid. The temperature was never allowed to go above 65° C. and usually was kept below 60° C. While the alcoholic extract did not give the results hoped, its method of preparation may be of some interest. The pancreas was ground up with sand and warm alcohol (40° C.) and extracted with enough alcohol to bring the total alcoholic percentage above 85 per cent. The glands were extracted for three twenty-four-hour periods at 35° to 40° C. This extract was then filtered and the filtrate evaporated to dryness under a negative pressure of about 70 cm. and in a slow stream of carbon dioxide, and the residue extracted with ether at room temperature. The ether extract was discarded and the residue dissolved in 95 per cent alcohol, and sufficient alcohol added to make up to such a volume that 10 c.c. of the solution represented 1 gm. of the fresh gland. For use, such a quantity as was desired was again evaporated as above and dissolved in 0.85 per cent sodium chloride solution for injection. The preparations when evaporated for the last time were of a light straw yellow and soluble with some difficulty in the sodium chloride solution, as much as 60 c.c. being required for the larger amounts of pancreas used.

In preparing the watery extract we sought to avoid the blood pressure lowering substances by rejecting all alcohol soluble material. The glands were prepared as in the first method, but after the alcohol had thoroughly penetrated the material it was evaporated to dryness under a vacuum and extracted for some time in 150 c.c. of absolute alcohol at 38° C. This was poured off as completely as possible and the residue extracted for one hour and twenty minutes with water rendered just acid with acetic acid. It was then filtered as rapidly as possible and evaporated as before. When dry, it was covered with absolute alcohol and kept for use. For injecting, the alcohol was poured off and 20 c.c. salt solution added. In appearance, when dry, it was almost the color of the first extract, possibly more collodial in appearance. It went into solution readily in the quantities of salt solution used, though the solution had somewhat the appearance of a finely divided colloid.

TABLE I.

Dog D.					
Date 1911.	Hour.	Urine.	Sugar.	D/N.	Remarks.
May		c.c.	gm.		
9	12.00 A. M.	...	...	...	Pancreatectomy completed.
10	10.45 A. M.	320	13.40	2.34	
11	11.00 A. M.	455	18.42	1.87	
12	11.00 A. M.	400	16.57	2.69	Injected the alcoholic extract from 5 gm. of fresh pancreas.
13	10.30 A. M.	285	13.77	2.72	
14	8.00 A. M.	395	17.07	2.79	
15	5.30 P. M.	...	...	...	Dead.

Methods of testing extracts. — Only dogs with pancreas completely removed are reported here. The complete removal was determined (*a*) by the constancy of the diabetic curve, and (*b*) by autopsy, though it was found that the first control was fully satisfactory. While only the significant portions of the tables are reported, we may say now, once for all, that the injections were made in each case after the sugar percentage in the urine had descended from the height occurring on the second or third day and had reached the level that is maintained more or less constant throughout the subsequent life of the dog. While it might have been advisable from some points of view to have secured more D/N ratios before the injections, it was assumed that when this had reached approximately 2.65 it had reached a fairly constant level.

RESULTS.

In brief, the results from the alcoholic extract were negative; the dogs in general showing an increase of D/N, though this in most

TABLE II.

Date 1911.	Hour.	Urine.	Sugar.	D/N.	Remarks.
DOG H.					
		c.c.	gm.		
June 30	2.30 P. M.	...	...	...	Pancreatectomy completed.
July 3	8.00 A. M.	265	7.20	2.68	For twelve hours only.
3	10.00 A. M.	...	...	...	Injected ex't of half a gland from a large dog. Calcu'd to be about the equival't to the pancreas of this dog.
3	9.00 P. M.	255	5.90	2.51	
4	10.55 A. M.	...	...	...	Second injection, as above.
4	2.15 P. M.	155	2.08	1.95	
6	6.45 A. M.	490	6.52	1.72	
6	5.15 A. M.	80	3.54	2.53	Dog in good condition.
7	5.30 A. M.	65	3.07	2.66	
DOG J.					
July 30	...	...	...	...	Pancreatectomy completed.
31	4.00 P. M.	307	14.61	2.92	
August 1	5.00 P. M.	626	20.71	2.60	
2	2.15 P. M.	471	14.91	2.53	
2	5.35 P. M.	...	...	...	Injected as in Dog H.
2	8.15 P. M.	90	2.37	2.14	
3	3.15 P. M.	305	8.92	1.93	
3	5.10 P. M.	...	...	...	Second injection as above.
4	5.00 P. M.	407	10.30	1.86	
5	2.00 P. M.	430	13.60	2.60	Dog in fair condition.

cases was not great enough to be significant. All the dogs showed great depression immediately following the injection and lasting in some cases for two or three hours. Several of the dogs died within two or three days following this injection. The tables are records of two typical cases.

Experiments with the watery extract were made on four dogs. The first two dogs and the fourth showed much the same type of D/N history. The third dog showed also a fall of this ratio following the injection, but the record was so irregular that the results were discarded. The first three dogs showed no depression following the injection and, if one dared say it, seemed even brighter for a time after the injection than before it. The fourth dog, in which an older extract was used, showed a marked depression lasting for an hour and a half.

SUMMARY.

Intravenous injections of the pancreas extract, prepared as above, into dogs rendered diabetic by complete pancreatectomy diminish temporarily the sugar excretion and lower the D/N ratio in the urine. It does not follow that these effects are due to the internal secretion of the pancreas in the extract. The injections are usually followed by a slight temporary rise of the body temperature, and this may be a factor in the lowered sugar output. Physiologists are not agreed as to whether the internal secretion acts by diminishing or retarding the passage of sugar from the tissues into the blood, or by increasing the oxidation of the sugar in the tissues. The pancreas extract may decrease the output of sugar from the tissues by a toxic or *depressor* action, rather than by a specific regulatory action of the pancreas secretion. If this is the case, we ought to get the same results by extracts of other tissues. The work is being continued in the hope of clearing up these points.

I wish to express my obligation to Dr. A. J. Carlson, who has offered helpful suggestions on the physiological side, and to Dr. W. Koch and Mr. F. C. Koch for assistance in preparing the extracts.

A STUDY OF CERTAIN TONIC AND REFLEX NERVOUS IMPULSES AS FACTORS IN PARATHYROID TETANY.

By H. J. MUSTARD.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

THE work here reported was undertaken, at the suggestion of Dr. A. J. Carlson, with the view of clearing up the question of the relation of certain "tonic" and reflex nervous impulses to the increased motor excitability, spasms, and tetany usually following removal of the parathyroid glands in the dog.[1] The work was done on dogs. Four series of experiments were performed:

1. The motor area of one side of the cerebral cortex was removed with simultaneous parathyroidectomy.
2. The motor area of each cerebral cortex was removed with
   (*a*) Simultaneous parathyroidectomy.
   (*b*) Later parathyroidectomy.
   (*c*) During tetany.
3. The spinal cord was transected in the upper thoracic region with simultaneous parathyroidectomy.
4. The posterior spinal nerve roots of one hind limb were cut, and the animals parathyroidectomized later.

The changes in the electrical excitability of the femoral nerve (left) to the galvanic current were recorded from time to time during the course of the disease. In those experiments in which the motor areas were removed in the first operation, the animal was given seven to ten days for recovery before the parathyroidectomy.

RESULTS.

The results of removal of the cortical motor area of one side with simultaneous parathyroidectomy are illustrated in the following extract of the protocol of a typical experiment:

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[1] The literature touching this problem is reviewed by BIEDL: Innere Sekretion, Berlin, 1910; and by CARLSON and JACOBSON: This journal, 1911, xxviii, p. 133.

June 21.		Dog. Motor area of left side removed; parathyroidectomy.
June 22.		No tremors, minimal cathodal closing contraction equals 1.5 milliamperes.
June 23,	11.00 A. M.	Respiration rapid and labored, tremors and tonic contractions in all parts of body posterior to neck. Twitchings of equal intensity on each side of body.
June 23,	3.00 A. M.	No tremors. Minimal Ca. C. C. = 0.5 M. A.
June 24,	8.30 A. M.	Reflexes good on both sides.
	2.40 P. M.	Violent tetany; respiration rapid, labored, salivation. Tetanic convulsions of equal intensity on both sides.
	5.30 P. M.	Intermittent tremors. Minimal Ca. C. C. = 0.3 M. A.
June 25.		No symptoms of tetany.
June 26,	9.00 A. M.	Depression, labored respiration, no tremors. Dog found dead at 12.00 M.

This group of experiments points to the conclusion that removal of the motor area of one cerebral cortex does not alter the tetany, either in character or intensity, occurring on the opposite side of the body. A marked hemiplegia was evident in the periods between the tetanic attacks. The previous observations of Horsley and Lang, Biedl and MacCallum, are thus confirmed.

Five dogs with cortical motor areas of both sides removed and with parathyroidectomy later exhibited very uniform course of the disease. The following protocol is typical:

July 13.		Dog. Cortical motor areas of both sides removed.
July 14–17.		Dog in good condition. Marked muscular weakness and motor paralysis.
July 18,	3.00 P. M.	Minimal Ca. C. C. = 2.0 M. A. Parathyroidectomy.
July 19.		No tremors, condition good. Minimal Ca. C. C. = 1.5 M. A.
July 20.		Slight tremors at 12.00 M. Vomiting after eating. Minimal Ca. C. C. = 0.6 M. A.
July 21,	11.00 A. M.	Slight tremors in all limbs, respiration slightly labored.
	6.00 P. M.	Minimal Ca. C. C. = 0.25 M. A.
July 22,	8.30 A. M.	Slight intermittent tremors in all limbs. Respiration slightly labored and more rapid than normal. Minimal Ca. C. C. = 0.05 M. A.
	5.30 P. M.	Distinct intermittent twitchings in all limbs. Spastic rigidity in limbs between contractions. Respiration slightly labored.

July 23.		No tremors; condition fairly good; eats. Minimal Ca. C. C. = 0.4 M. A.
July 24.		No tremors; apparent weakness; eats. Minimal Ca. C. C. = 0.6 M. A.
July 25,	8.30 A. M.	Minimal Ca. C. C. = 0.3 M. A. No tremors.
	11.00 A. M.	Tremors and convulsions in all limbs typical of latter stages of tetany. Tetanic spasms of equal intensity in all limbs and in muscles of head. Marked opisthotonus; salivation; diarrhea.
	5.30 P. M.	No convulsions; spastic rigidity. Minimal Ca. C. C. = 0.6 M. A.
	5.37 P. M.	Dog died (not in tetany).

The five experiments showed no deviation from the usual symptoms which could not very easily be ascribed to individual variations. No essential difference could be seen between the course of the disease in these dogs and in the dogs with the motor cortex and the parathyroids removed simultaneously. Our results thus confirm the earlier observations of Biedl.

In two experiments the motor areas of each cerebral hemisphere were removed while the dogs were in tetany. A summary of one of the protocols follows:

June 26.		Dog. Minimal Ca. C. C. = 1.8 M. A. Parathyroidectomy.
June 27.		No tremors. Minimal Ca. C. C. = 0.6 M. A.
June 28,	8.30 A. M.	No tremors. Minimal Ca. C. C. = 0.25 M. A.
	2.00 P. M.	Respiration rapid and labored. Tremors in head and shoulders.
	5.00 P. M.	Tremors over entire body, with distinct twitchings in head muscles and in fore limbs. Cortical motor areas of both sides removed.
	6.30 P. M.	Tetanic convulsions appear on recovery from ether.
	7.30 P. M.	Twitchings very marked in head, neck, and fore limbs. Slight tremors in lower part of back.
June 29,	8.30 A. M.	No tremors.
	10.00 A. M.	Minimal Ca. C. C. = 0.8 M. A.
	12.00 M.	Slight tremors and twitchings. Respiration slightly labored. Vomiting and diarrhea.
	5.30 P. M.	Dog same. Minimal Ca. C. C. = 0.15 M. A.

June 30,	8.30 A. M.	Respiration slow and labored. Tetanic convulsions over entire forepart of the body. Vomiting and diarrhea. Minimal Ca. C. C. = 0.1 M. A.
	9.07 A. M.	Dog died in tetany.

In this group of experiments the possibility of the functions of the motor areas being assumed by other centres was eliminated, for only after several hours, at least, could such an adaptation be established. The other dog gave closely corresponding results. It is evident, then, that the tremors and contractions of tetany do not depend primarily upon stimuli coming from the motor cortex or on "tonic" influences exerted by the cortical centres by way of the motor areas.

Attention is now invited to the series of five dogs with high thoracic transection of the spinal cord and simultaneous parathyroidectomy. The animals showed very little variation in symptoms. The following protocol is typical:

June 26,	12.00 M.	Dog. Minimal Ca. C. C. = 1.5 M. A. Parathyroidectomy. High thoracic transection of the spinal cord.
	5.30 P. M.	Minimal Ca. C. C. = 1.00 M. A. Reflexes in posterior part of dog good.
June 27.		No tremors. Minimal Ca. C. C. = 0.4 M. A.
June 28,	8.30 A. M.	No tremors. Minimal Ca. C. C. = 0.3 M. A.
	2.30 P. M.	Tremors and slight twitchings in anterior part of dog. Very slight tremors in lower part of back and gluteal muscles.
	5.00 P. M.	Tetany anterior to transection very severe. Respiration rapid, labored. Only slight tremors in hind limbs.
	8.00 P. M.	Respiration labored. No tremors or twitchings. Minimal Ca. C. C. = 0.9 M. A.
June 29.		No tremors or tetany. Minimal Ca. C. C. = 1.3 M. A.
June 30,	9.00 A. M.	Dog greatly depressed, died while being handled.

These results accord with the observations of Horsley and of Carlson and Jacobson. The epileptic convulsions and tetany do not extend to the part of the animal posterior to the spinal transection, despite the increased motor excitability and augmented reflexes. The tetanic attacks are therefore conditioned on some part of the encephalon below the cerebral cortex. The "spontaneous" contractions that sometimes appear posterior to the spinal transections during the epileptic attacks in the anterior part of the dog are probably explained correctly by Carlson and Jacobson as reflexes initiated by the pressure on the viscera and tension from muscular contractions anteriorly.

It is well known that section of the posterior spinal roots in the dog diminishes the reflex excitability of that region of the spinal cord.[2] What is the effect of such a lesion on the course of parathyroid tetany? We present one typical protocol of a dog with the posterior roots of the lumbar nerves on one side cut, and the animal parathyroidectomized later:

Aug. 3.		Dog. Left posterior roots of lumbar nerves cut by Dr. F. C. Becht.
Aug. 4.		Left hind leg hangs limp. No response to pain stimuli except in gluteal region.
Aug. 5,	4.30 P. M.	Minimal Ca. C. C. = 1.5 M. A. Parathyroidectomy.
Aug. 6.		No tremors. Minimal Ca. C. C. = 1.5 M. A.
Aug. 7,	8.30 A. M.	Tremors and twitchings in head and anterior part of body. Hind limbs show only slight twitchings of equal intensity in each limb.
	2.30 P. M.	Tremors.
	10.00 P. M.	No tremors.
Aug. 8.		No tremors. Minimal Ca. C. C. = 0.5 M. A.
Aug. 9,	12.00 M.	Respiration labored. Tremors very marked. Twitchings in all limbs, and in head and neck. Twitchings of equal intensity in both hind limbs.
	4.00 P. M.	Symptoms same. Experiment discontinued.

The other experiments of the series ran a similar course. The symptoms of parathyroid tetany are quite as severe in a limb with all sensory nerves cut as in a normal limb. The increase in the direct excitability of the motor neurones and the tremors and tetanic attacks are not appreciably retarded or modified by section of the afferent nerves.

SUMMARY.

1. The rate of development and the final degree of the increased excitability of the motor neurones following parathyroidectomy in

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[2] BROOKS: This journal, 1910, xxvii, p. 212.

the dog are not appreciably influenced by ablation of the cerebral motor cortex, spinal transection, or section of the dorsal nerve roots. The increased excitability is therefore probably due primarily to some direct chemical action on the motor neurones.

2. The course of the parathyroid tetany is not appreciably influenced by ablation of the motor cerebral cortex, or by rendering a limb apastic through section of its afferent nerves.

3. As the epileptic spasms or tetanic attacks following removal of the parathyroid glands do not develop posterior to the spinal transection, it would seem that the actual tetany depends, not only on the local increase of motor excitability in the spinal cord, but also upon nervous connections with some region of the encephalon below the cerebral cortex.

I wish to thank Dr. Carlson for his constant direction, assistance, and criticism.

MICROSCOPIC STUDIES OF LIVING SMOOTH MUSCLE.

By EDWARD B. MEIGS.

[From the Wistar Institute of Anatomy and Biology.]

THE comparison of fixed specimens of uncontracted and contracted smooth muscle points strongly to the view that the cells of this tissue decrease in volume during contraction. In the preparations of contracted muscle the fibres are no thicker than in preparations of uncontracted muscle; the shortening of a fibre without any increase in its thickness would of course mean a considerable decrease in its volume. The spaces between the fibres are much larger in the preparations of contracted muscle, which indicates that the decrease in volume of the fibres is the result of a transfer of fluid from them to the spaces between them.

It has been reported by Schultz,[1] however, that the living smooth muscle cells may be seen to grow thicker as they contract beneath the microscope, and this assertion raises the question whether the fixed preparations can be regarded as trustworthy evidence for what goes on in the living muscle during contraction. I have made further studies both of fixed preparations and of living tissue, and have endeavored to determine whether contraction really is accompanied by a change in the volume of the muscle cell or not. I shall consider first the evidence to be obtained from the fixed preparations, and then go on to what may be learned from a study of the living tissue.

Observations on fixed preparations. — Heiderich reports[2] that in fixed preparations the contracted cells usually have a less cross sectional area than the uncontracted ones. The interstitial spaces in the contracted muscle are larger, and the contracted cells stain darker and more homogeneously than the uncontracted ones. The nuclei of the contracted cells, however, are not only shorter but also thicker than those of the uncontracted ones. I have made studies more or less similar to those of Heiderich,[3] and have confirmed his results in every respect, except that in my specimens the contracted fibres usually had about the same diameter as the uncontracted ones. These observations could be explained by supposing that during the contraction of smooth muscle the nuclei maintain the same volume, but that the cell bodies lose fluid which passes to the interstitial spaces.

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[1] SCHULTZ: Archiv für Physiologie, 1895, pp. 530, 531.

[2] HEIDERICH: Anatomische Hefte, 1902, xix, p. 451.

Heiderich gives a different interpretation to his results. He believes that the contracted cells shrink more under the influence of histological treatment than the uncontracted ones, and he brings some evidence to the support of this view. Whether or not his evidence is regarded as of any value, it is clearly highly desirable that studies should be made on the contraction of living smooth muscle, and I have, therefore, turned my attention to this side of the subject.

The changes undergone by living smooth muscle cells during contraction. — Schultz has already studied the contraction of living smooth muscle cells,[4] using a longitudinal strip of the stomach muscle of Salamandra maculosa. He states that the cells of this tissue become broader during contraction. I have spent some time in trying to repeat Schultz's observation on a longitudinal strip of the stomach muscle of the frog.

If a thin section of frog's stomach muscle is cut off according to the method described by Schultz and examined under the microscope in Ringer's solution,[5] the muscle nuclei and the outlines of the cells can be seen. Further, such pieces of muscle readily contract when stimulated by an electric current, and give during contraction the general impression that the cell outlines move further apart. But the cells of amphibian stomach muscle lie very close together; hence a longitudinal section cannot be made thin enough to give a single layer of cells with open spaces between them; one always sees the whole field occupied by cell bodies, separated by narrow lines which appear bright or dark according to the focus. It is always impossible to say whether a pair of neighboring outlines belong really to the cell body which lies between or to overlapping neighboring cells.

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[3] Meigs: This journal, 1908, xxii, p. 477.

[4] Schultz: Archiv für Physiologie, 1895, pp. 530, 531.

[5] By Ringer's solution in this article is meant a solution with the following formula: NaCl, 0.65 parts; KCl, 0.02 parts; $CaCl_2$, 0.025 parts; $NaHCO_3$, 0.02 parts; H_2O, 100 parts.

This is true, even when the stationary material is examined. But during contraction the muscle becomes thicker; the part under observation is pushed up closer to the objective and out of focus; and the observer gets only the most confused idea of what occurs to any given pair of outlines.

Of course the observer would get the general impression that the cell outlines moved further apart during contraction, whether the individual cells became thicker or whether they remained of the same width and were merely pushed further apart from each other. And if the observation were made with the strong belief that smooth muscle cells become thicker during contraction, it would naturally be assumed that any pair of neighboring outlines which happened to move apart did actually belong to the cell body which lay between them and not to the overlapping neighboring cells.

The way in which presuppositions as to what ought to occur may mislead even the best observers is well illustrated by the mistake into which Engelmann was led. Engelmann found that in fixed specimens of contracted striated muscle the volume of the anisotropic substance had increased at the expense of that of the isotropic substance.[6] He then turned to the observation of living muscle, and reported that in this the anisotropic bands increased at the expense of the isotropic bands during contraction;[7] and for many years no one of importance contradicted him. Recently, however, Hürthle has succeeded in taking cinematographic photographs of living contracting muscle,[8] and these show very clearly that during contraction the isotropic bands increase at the expense of the anisotropic bands — exactly the opposite of what Engelmann thought he observed.

In view of these considerations, and of the great difficulty in seeing what occurs in such preparations of smooth muscle as Schultz used, I have sought for an object in which the changes undergone by living smooth muscle during contraction could be demonstrated more satisfactorily. I have experimented with strips of the frog's bladder, with the mesentery of Desmognathus fusca,[9] and with fresh cross sections of frog's stomach muscle. The frog's bladder is not a more favorable object for the examination of living smooth muscle cells than the stomach. But significant results have been obtained with the other preparations mentioned, and these will now be described.

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[6] ENGELMANN: Archiv für die gesammte Physiologie, 1878, xviii, p. 1.

[7] ENGELMANN: Nature, 1895, li, p. 523.

[8] HÜRTHLE: Archiv für die gesammte Physiologie, 1909, cxxvi, p. 1.

[9] Desmognathus fusca is one of the small urodelous amphibians.

Experiments on the mesenteric muscle of Desmognathus fusca. — Certain structures in the mesentery of Desmognathus fusca have the appearance of typical smooth muscle cells, and are stained by nigrosin and picric acid in a way that is characteristic for smooth muscle. Occasionally isolated cells can be found. Careful study of fixed preparations, however, shows that such instances are rare; it is more common for three or more cells to be bundled together, and in the fresh tissue it is very difficult to determine how many cells compose a bundle. The individual cells are rather small — seldom more than three or four micromillimetres in diameter.

I have found it difficult to make these cells contract, though I have used strong galvanic and rapidly interrupted Faradic currents which produced marked contractions in the stomach muscle of Desmognathus. On one occasion application of the current was followed by the drawing away of the nucleus of one of the cells from some neighboring tissue, and I could not see that the cell body on the side toward which the nucleus was drawn became any thicker. Such an observation, however, is of very little value for several reasons. Even if the cell shortened to half its original length, which is rare as a result of electrical stimulation, the increase in diameter necessary in order that it might retain its original volume would be only from 1 to 1.414. It would be difficult to see so small an increase in diameter in an object so small and with such dim outlines as a smooth muscle cell. Further, one could not be sure that the part of the cell under observation was actually contracting; it might be that the pull was the result of the contraction of tissue outside the field of vision. Under these circumstances, of course, the part of the cell under observation could not be expected to become thicker.

In the hope of making the fresh cells more readily visible, I have tried to stain pieces of the mesentery and whole animals with methylene blue and neutral red. All my attempts in this direction, however, have been complete failures. The cells stain so faintly and so little darker than their surroundings that their visibility is not appreciably increased.

Nevertheless, I have been able to make certain observations on the mesenteric smooth muscle cells, which may be of considerable interest. It has been observed both by Heiderich and by me that in fixed specimens of smooth muscle the contracted cells differ from the uncontracted ones in the relations which obtain between the cell bodies and the nuclei. In the uncontracted cells the nuclei are long and narrow, and the cells are little or not at all wider at the nuclear regions than at others. In the contracted cells, on the other hand, the nuclei are shorter and thicker than in the uncontracted ones, and the cells are bulged out in their neighborhood. If the cells are measured at the middle of the nuclei, it is found that the contracted ones are nearly twice as thick as the uncontracted, but at distances of about 10 μ from the ends of the nuclei the contracted cell bodies have the same thickness as the uncontracted ones. Figure 1 is a rough representation of these relations.

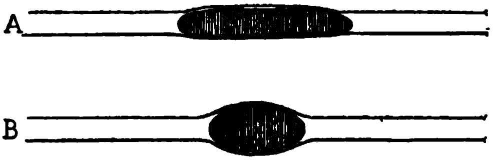

FIGURE 1. — Diagrammatic drawing to show the differences between relaxed and contracted smooth muscle cells, as they appear in the nuclear regions. *A*, relaxed; *B*, contracted.

The whole appearance is such as to suggest that while the cell bodies become shorter during contraction without becoming thicker, the nuclei become both shorter and thicker, and presumably therefore maintain the same volume through all stages of contraction.

But if Heiderich's interpretation of the phenomena be accepted, it must be supposed that Fig. 1, *B* does not accurately represent the form of a living contracted cell. Heiderich thinks that the cell bodies of contracted muscle shrink more than those of uncontracted muscle, and that this is the reason why the former are not broader than the latter in fixed preparations. He would have to suppose, therefore, that the relations which obtain between the diameters of cell body and nucleus in *B* are not the result of contraction alone, but of contraction followed by fixation and embedding.

If the fresh mesentery of Desmognathus fusca be examined in Ringer's solution, some cells are found with long narrow nuclei like that of *A*, Fig. 1, and others, with short thick nuclei like that of *B*, Fig. 1. It seems reasonable to suppose that the former are uncontracted, and the latter contracted. If the cell bodies at distances of 10 μ from the ends of the nuclei are compared, they will be found to have the same diameter in both cases; the cell bodies with the short thick nuclei are not appreciably wider than those with the long narrow nuclei; the former are bulged out in the nuclear regions just like the fixed contracted cells. The study of the muscle cells in the mesentery of Desmognathus fusca, therefore, indicates that the cell bodies of smooth muscle decrease in volume during contraction.

Experiments on fresh cross sections of frog's stomach muscle.—While endeavoring to repeat Schultz's observation on fresh stomach muscle, I happened to find some slices in which the fibres were cut transversely. In such preparations I could see the boundaries between the fibres and the interstitial spaces comparatively clearly, and I got a much better idea of the relative volumes than in longitudinal sections. Recent experiments, of which the results are still unpublished, have convinced me that cutting across the fibres of smooth muscle does not destroy the irritability even in the immediate neighborhood of the cut. I have found that slices of smooth muscle, in which the individual fibres had a length of only two or three millimetres between cuts, remained highly irritable for many hours in Ringer's solution at room temperature. I have therefore stimulated cross sections of fresh stomach muscle[10] while subjecting them to microscopic observation, and have found that they often do contract. I have the impression that contraction is accompanied by an increase in the relative volume of the interstitial spaces. But the change of focus and the gross movements which occur as a result of contraction make it difficult to see the finer changes, and, as I realize my strong prejudice in favor of the view that the interstitial spaces become relatively larger during contraction, I have sought a method of experimentation by which the differences between relaxed and contracted fresh preparations of smooth muscle could be studied more satisfactorily.

It is easy to throw smooth muscle into a state of maintained contraction or tone; immersion in slightly alkaline solutions has this effect on the tissue. In the experiments to be described I have used

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[10] The form of stimulation used in this experiment and in the experiments to be subsequently described in this article was a moderately strong rapidly interrupted Faradic current.

Ringer's solution to which a little Na_2CO_3 has been added.[11] Smooth muscle immersed in this fluid goes into a state of tone which is maintained for several hours. In an hour or so it loses its power to contract in response to an electrical stimulus, but regains its irritability on being washed out thoroughly with neutral Ringer. Thin cross sections of fresh smooth muscle immersed in alkaline Ringer lose their power of responding to electrical stimulus in fifteen or twenty minutes, but regain it if afterward washed out for the same length of time in neutral Ringer.

The experiments with the alkaline Ringer solution were carried out as follows. The mucous and muscular layers of a frog's stomach were separated from one another; two strips were cut from the muscular layer, and of these one was immersed in neutral Ringer and the other in alkaline Ringer.[12] It has been determined experimentally that pieces of smooth muscle usually tend to relax in neutral Ringer. Three or four free-hand transverse sections were cut off from each strip with a sharp razor, and each slice was mounted on a separate slide. The slices from the strip which had been in neutral Ringer were, of course, mounted in neutral Ringer, and the others, in alkaline Ringer. The sections so made were found to be from a hundred to two hundred micromillimetres in thickness. It is well to support the cover slips of the preparations with small strips of filter paper.

The six or eight slides so obtained were mixed up, and each was then examined under the microscope without my knowing whether it held a slice of muscle in neutral or in alkaline Ringer. I endeavored to decide in which sections the interstitial spaces were larger in relation to the area occupied by the sections of the fibres. The three or four slides in which the interstitial spaces were larger were separated from the other three or four in which they were not so large, and then the fluid under the cover-slip of each was tested with litmus paper to see whether or not it was alkaline. There was thus obtained an unprejudiced judgment as to whether the contraction produced in smooth muscle by alkaline Ringer is accompanied by an increase in the relative volume of the interstitial spaces. Such experiments were tried with twenty-eight pairs of sections in alkaline and neutral Ringer, and the contracted were separated from the uncontracted correctly in twenty-four cases; I failed to separate them correctly in the remaining four.

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[11] The solution in question was made by mixing 9 parts of Ringer solution with 1 part of 1.3 per cent Na_2CO_3 solution. The addition of the Na_2CO_3 is followed by the precipitation of $CaCO_3$, which is filtered off. The solution so obtained differed, of course, from Ringer's solution in having little or no calcium as well as in being alkaline, but it will be called, for the sake of brevity, simply "alkaline Ringer."

[12] The muscle to be used in these experiments should be taken fresh from a recently killed frog, or kept before the experiment in a moist chamber; it should not be kept for any length of time before the beginning of the experiment in Ringer's solution. Smooth muscle often takes up considerable quantities of fluid from Ringer's solution, and this interferes with the results to be obtained.

DISCUSSION.

The experiments which have just been described were carried on partly in June and July and partly in September and October at temperatures which varied between 18° and 30° C. The frogs experimented on were for the most part bull-frogs, but a few of the experiments were carried out on the muscle of the green frog. The four failures did not occur under any particular set of conditions, but were scattered along fairly evenly through the whole series of experiments, and I feel confident that the occasional failure of this kind of experiment is not to be attributed to the influence of season, temperature, or species of frog used.

But if sections of fresh muscle, made as nearly as possible under similar conditions, and mounted in neutral Ringer be compared, it will be found that there is often considerable variation in the relative volumes occupied by the fibres and the interstitial spaces. It was thought at first that this variation might be only apparent and due to the impossibility of cutting free-hand sections of uniform thickness — that the interstitial spaces might appear larger in the thinner sections. But experimentation with sections which were purposely cut of widely different thickness showed that the thickness of the sections makes relatively little difference in the apparent size of the interstitial spaces. It is probable that the varying size of the interstitial spaces in sections prepared as nearly as possible in a similar manner is the expression of the fact that in some of these sections the muscle is more contracted than in others. It is well known that smooth muscle is a tissue which is rather capricious in its reactions;

if a number of rings be cut from the stomach of the same frog and placed under similar conditions in Ringer's solution or in a moist chamber, it will be found that the tone of some of the rings increases, while that of others decreases. It seems highly probable that small uncontrollable differences in the manner of holding and cutting the muscle may result in some parts of it being more contracted than others.

The fact that sections of muscle treated in the manner above described with neutral Ringer sometimes show relatively large interstitial spaces explains why a small proportion of the experiments under consideration have been failures. It remains to describe in a little more detail the results of the comparison which has been carried out.

After making certain that the treatment with alkaline Ringer has the effect of making the interstitial spaces relatively larger, I made careful studies of the two kinds of sections with the view of determining a number of other points. The determinations of the relative sizes of the interstitial spaces in the experiments which have just been described were made as soon as possible after the sections were cut, for I wished to be sure that the tissue I was examining was still living. But I have found that the differences in the relative sizes of the interstitial spaces in the sections treated with neutral and alkaline Ringer respectively become more marked with the passage of time; I believe the failures would have been fewer if I had examined the sections an hour or so after they had been mounted. Strips of muscle immersed in alkaline Ringer often continue to shorten for an hour; it is probable that the sections behave in the same way, and that this is the reason why they show relatively larger interstitial spaces an hour after they have been mounted.

The differences between the contracted and uncontracted sections are often very marked. The interstitial spaces appear to vary from about a tenth of the total volume of the muscle to about a third. But this judgment must be regarded as rough, for the sections are far from being satisfactory histological preparations. The thickness of the sections and the slightness of the difference in opacity between the fibres and the interstitial spaces make accurate observation difficult.

I have attempted to measure the diameter of the fibres and the size of the interstitial spaces in the relaxed and contracted sections by means of the eye-piece micrometer. But the task is one of great difficulty. The fibres vary greatly in size and are more or less irregular in shape, and the introduction of the micrometer makes accurate observation even more difficult. The measurements indicate the same conclusions as direct observation, and show besides that there is no marked difference in the diameter of the contracted and uncontracted fibres. But I regard the evidence from the measurements as less convincing than that from direct observation.

It is to be noted that the question whether the fibres increase in diameter during contraction is more or less distinct from the question whether they decrease in volume during contraction. If it could be shown that the fibres shorten without becoming thicker, it would, of course, follow that the shortening was accompanied by a decrease in volume. But if it were shown that the fibres did increase in thickness during contraction, it would not necessarily follow that they did not decrease in volume; the question would be whether the increase in thickness was enough to compensate for the decrease in length. It is conceivable that the fibres might become slightly thicker during contraction, but not enough to make up for the decrease in length.

It would be an almost impossible matter to determine from fresh sections of smooth muscle whether the contracted fibres had a slightly greater or less diameter than the uncontracted ones, and no time has been spent in the attempt to make this determination. The main object has been to determine the question whether the fibres decrease in volume. The greater relative size of the interstitial spaces in the contracted sections shows that the fibres have decreased in volume, provided it can be shown that the fluid which fills the larger interstitial spaces of the contracted sections has come from the fibres and not from the fluid in which the sections are immersed. Otherwise it might be thought that alkaline Ringer had the property of making the interstitial spaces of the tissues swell more rapidly than the fibres. The appearance of sections which had been treated with the reagent could then be explained by supposing that the fibres had remained of the same volume while the interstitial spaces had increased.

The changes in weight undergone by smooth muscle in alkaline Ringer have been followed and it has been found that such preparations as I have ordinarily made do undergo a slow increase in weight —

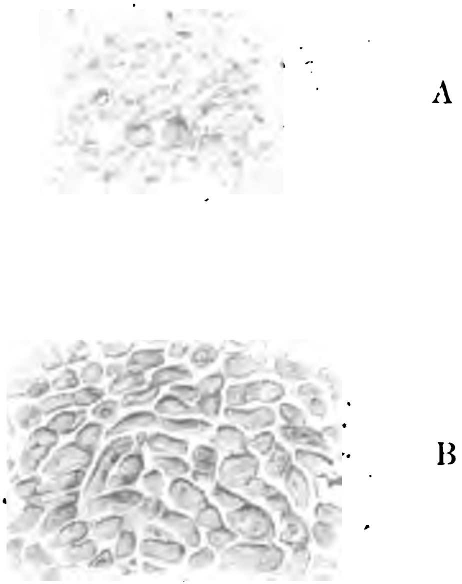

Figure 2. — Drawings to show the differences in appearance of fresh transverse sections of smooth muscle treated with neutral Ringer and with alkaline Ringer respectively. *A*, section treated with neutral Ringer; *B*, section treated with alkaline Ringer. Amplification, 375 diameters.

about 10 per cent in the course of an hour. This increase is less than often occurs in neutral Ringer.

My ordinary preparations of smooth muscle contain about 15 per cent of sub-mucous and serous connective tissue, and I have found this much swollen in preparations which have been immersed in alkaline Ringer. It may be shown also that the connective tissue of tendon undergoes a considerable swelling when immersed in alkaline Ringer. If a piece of stomach muscle be stripped of connective tissue as far as can be done without seriously injuring the muscle, it will be found that it swells considerably less in alkaline Ringer than does the ordinary preparation. It may be said, therefore, that if smooth muscle *per se* swells at all in alkaline Ringer, the swelling is far too small to account for the increase in the volume of the interstitial spaces which can be demonstrated microscopically, and it must be supposed that immersion in this reagent causes fluid to pass from the fibres to the interstitial spaces.

I have been anxious to publish with this article illustrations which should give an idea as exact as possible of the difference in the appearance of sections of smooth muscle treated with alkaline Ringer and with neutral Ringer respectively. I have therefore tried to have camera lucida drawings made of the contracted and uncontracted sections, but the fibres are so little darker than the interstitial spaces that they cannot be distinguished in the face of the light reflected from the paper by the camera. Microphotographs of the sections are not sufficiently satisfactory to be worth publishing. But I have had drawings traced from photographs by an artist who has combined the study of the photographs with that of the sections themselves, and these give a very good idea of the differences which are commonly found. Such drawings are reproduced in Fig. 2. They show certain interesting points which can be made out by a careful study of the sections. The contrast between the opacity of the fibres and that of the interstitial spaces is sharper in the sections treated with alkaline Ringer; while the contrast between the nuclei and the fibres is rather sharper in the sections treated with neutral Ringer. Similar differences can be shown in sections of muscle fixed in relaxation and contraction by Zenker's fluid; they are probably merely another expression of the fact that during contraction fluid passes from the fibres to the interstitial spaces, leaving the colloids of the former more concentrated and rendering the latter more watery; and they furnish further evidence for the view that the fixed sections give a true history of what occurs during contraction.

There are other reagents besides alkaline Ringer, in which smooth muscle goes into a state of marked contraction, and in which it remains alive for some time. Ringer's solution to which small quantities of $BaCl_2$ have been added has this effect. I have tried a few experiments with a mixture containing three parts of Ringer's solution and one part 2.2 per cent commercial $BaCl_2$; this mixture has about the same osmotic pressure as Ringer's solution, and strips of smooth muscle immersed in it go into a state of marked contraction. Transverse sections of fresh smooth muscle treated with this mixture show an increase in the relative volume of the interstitial spaces. This increase is most marked if the strips of muscle, from which the sections are to be cut, are left for five or ten minutes in the mixture before cutting.

But sections which have been treated with the mixture of Ringer and $BaCl_2$ show a peculiar dimness in the outlines of the fibres and a characteristic shading of the surface of the section. These peculiarities make it easy to distinguish these sections from others treated with pure Ringer without reference to the relative areas occupied by the fibres and the interstitial spaces, and I have, therefore, not carried out a series of experiments with the $BaCl_2$ mixture similar to that described above, in which sections treated with neutral and alkaline Ringer respectively were compared with each other. The mixture of Ringer and $BaCl_2$ causes in the first hour or so a slight decrease in the weight of pieces of muscle immersed in it.

A Ringer's solution in which all the salts have double the usual concentration is an example of another class of reagents which cause smooth muscle to go into a state of marked contraction. Pieces of smooth muscle immersed in double strength Ringer shorten fairly rapidly and remain shortened, and, at the same time, decrease considerably in weight. If sections of fresh smooth muscle treated with double strength Ringer be compared with other sections treated with ordinary Ringer, it will be found that the interstitial spaces of the former are relatively as large or larger than those of the latter, and it is evident therefore that in this case also the fibres lose volume at the same time that they contract. The interstitial spaces in the muscle treated with the double strength Ringer appear darker than those of the muscle treated with the ordinary Ringer.

SUMMARY.

In fixed preparations of smooth muscle the contracted cells have about the same diameter as the uncontracted cells except at the nuclear regions; the nuclei of the contracted cells are thicker. If the fresh mesentery of Desmognathus fusca be examined in Ringer's solution, it will be found that some of the muscle cells have long thin nuclei, and others short thick nuclei; the cell bodies, however, have in both cases the same diameter.

In fixed preparations of smooth muscle the interstitial spaces are larger in proportion to the volume of the fibres in the contracted muscle; the same relation can be demonstrated by comparing fresh sections of smooth muscle in Ringer's solution with others that have been caused to contract by treatment with slightly alkaline Ringer and other fluids. In other words, the main points of difference between relaxed and contracted muscle in fixed preparations, which indicate that the fibres lose volume during contraction, can be demonstrated in fresh tissue also. It may, therefore, be concluded that, during the contraction of smooth muscle, fluid passes from the fibres to the spaces between them; there is no reason to believe that relaxed and contracted muscle fibres react differently to histological preparation, as Heiderich has supposed.

THE SEPARATION OF RENNIN AND PEPSIN BY THE PASSAGE OF A DIRECT ELECTRIC CURRENT.

By W. E. BURGE.

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IN spite of the many publications devoted to the subject there is still a difference of opinion among recent writers in regard to the specificity of rennin. The original and natural view proposed by Hammarsten,[1] according to which rennin and pepsin are separate and distinct enzymes, has been contested, as is well known, by a number of writers, and especially by Pawlow. The fact that a rennet action is exhibited by proteolytic enzymes wherever found, even among the plants and bacteria and in other locations in which there can be no question of a functional value so far as the digestion of milk is concerned, has tended perhaps to strengthen the views of those who hold that the coagulation of milk is not effected by a specific enzyme. But even among those who hold this view there is a divergence of opinion in regard to the relationship between the rennin and the protease with which it is associated. While some have considered that pepsin, for example, and rennin are identical, that is to say, that the rennetic action is due to the pepsin, others have adopted the suggestion made by Nencki and Sieber according to which the peptic and the rennetic actions are due to separate side-chains of the same molecule, while Vernon [2] advocates the view that rennin is a mere by-product in the formation of proteolytic enzymes and has no functional value. A review of the current literature upon this subject makes very plain, in fact, that the existing divergence of opinion can only be reconciled by accumulating further evidence.

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[1] HAMMARSTEN: Maly's Jahresberichte, 1872, i, p. 118, and also Zeitschrift für physiologische Chemie, 1908, lvi, p. 18. For full literature consult this latter article, as also FULD: Ergebnisse der Physiologie, 1902, i, part i, p. 468.

[2] VERNON: Intracellular enzymes, London, 1908, p. 157.

With this need in mind the present investigation was begun to determine what effect, if any, the passage of a direct electric current of low amperage may have on a solution which both digests protein and curdles milk.

Two specimens of commercially prepared rennin were used. Either of these would digest pigs' fibrin as well as curdle fresh cow's milk. For the purposes of this investigation no difference was found in the two commercial preparations.

The determinations of peptic power were made by means of a fibrin digestion, and of the rennetic power by the time necessary for curdling fresh cow's milk.

The fibrin used was prepared in the following way: Fresh pig's fibrin was washed free of blood and heated in water at 95° C. for five minutes. It was then removed, squeezed free of the excess of water, and placed in 25 per cent alcohol. When ready for use, a piece was taken from the alcohol, washed thoroughly with distilled water, and cut into small pieces. These pieces were pressed between folds of filter paper to remove as much water as possible. Each piece was then weighed.

Seven grams of the commercial specimens of rennin were dissolved in 300 c.c. of water, to which were added 10 c.c. of 0.6 per cent hydrochloric acid, and four crystals of thymol. To test the fibrin-digesting power of this solution 2 c.c. were poured into a test tube, made neutral to litmus by the addition of a 0.2 per cent solution of sodium bicarbonate, and then acidified by the addition of 8 drops of 1 per cent hydrochloric acid. Into this acidified solution was put a piece of pig's fibrin, prepared and weighed, as described above. The test tube, containing the acidified solution and piece of fibrin of known weight, was placed in a thermostat at 38° C. The time required for dissolving such a piece of fibrin weighing approximately 200 mg., at 38° C., was five to six hours. At this time the clear solution with which the experiment was begun had become a little turbid and the piece of fibrin had gone into solution, leaving only very small particles of insoluble material. This solution was tested for peptone by the biuret reaction, which was positive in all cases.

The milk-curdling power of the solution was determined in the following manner: One cubic centimetre of the solution was placed in a test tube, neutralized by adding a few drops of a 0.2 per cent solution of sodium bicarbonate, and acidified with two drops of 0.2 per cent hydrochloric acid. One cubic centimetre of fresh cow's milk was added to the acidified liquid, and the tube placed in a beaker containing water at 38° C. The time of clotting was thirty-five to forty-five seconds. Control experiments showed that the degree of acidity used did not cause in itself any clotting.

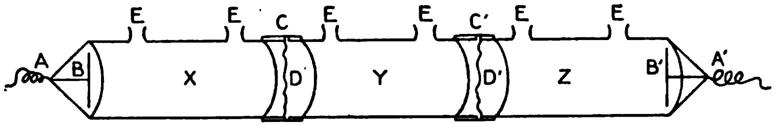

FIGURE 1. — *x*, *y*, and *z* represent three glass tubes held together by means of two rubber cuffs, *c* and *c'*. *d* and *d'* are two membranes of goldbeater's skin stretched over the ends of the middle tube, *y*. *a* and *a'* are two platinum wires fused into the ends of the two outer tubes, *x* and *z*, and attached to two platinum disks, *b* and *b'*. The *e*'s represent openings into the different tubes. The length of the whole tube, made up of the three tubes *x*, *y*, and *z*, is 60 cm. The inside diameter is 4 cm., and the diameter of each electrode is 3 cm.

At least three parallel determinations were made, both for the time of digesting a known amount of protein and for the time of curdling 1 c. c. of fresh cow's milk. All these determinations were carried out under perfectly constant conditions as to acidity of media, temperature, etc.

The problem now was to determine what effect the passage of a direct electric current of low amperage would have on such a solution in which the peptic and rennetic actions took place in the time stated.

In solving this problem eight determinations were made, each of which was carried out as follows: Two hundred cubic centimeters of a solution of commercial rennin, prepared as previously described, were electrolyzed in a tube, the construction of which may be understood by referring to the diagram representing it in longitudinal section (Fig. 1).

Two hundred cubic centimetres of rennin solution were poured into the inner tube, *y*. The two end tubes, *x* and *z*, were filled with distilled water to which were added a few drops of 0.2 per cent hydrochloric acid. A crystal of thymol was put into each of the three tubes. The tube was then connected across the electrodes of an electric circuit in series with a potential reducer and a milliammeter. In this way the resistance could be so regulated as to permit ten milliamperes of current to pass through the solution for twenty-five hours. With this amount of current passing no change in temperature could be observed with an ordinary centigrade thermometer. After the current had been passing for an hour or more, the liquid around the anode, as would be expected, became acid in reaction, and that around the cathode alkaline. The solution in the middle tube, *y*, was always slightly acid in reaction to litmus and to phenolphthalein. The exact degree of acidity may be indicated by the fact that one drop of the solution required two drops of a 0.2 per cent solution of sodium bicarbonate for complete neutralization.

From time to time during the twenty-five hours while the current was passing through the solution, tests were made of the protein-digesting and milk-curdling power of the liquid from different parts of tube *y*. These samples could be easily obtained by means of a small pipette introduced through the openings *e*, *e*, in the tube.

The effect produced on the peptic and rennetic power of the solution in the middle tube, *y*, is all that is to be considered in this article. The effect produced in different parts of the tube is now being investigated.

The quantities of the solution used in testing for the presence of rennin and pepsin were the same as those used before electrolyzing the solution, and the procedure was identical, three parallel tests being made in all cases. After the current had passed for twenty-five hours it was found that the liquid in tube *y* had lost completely its peptic activity. The specimen of fibrin, after remaining in the warm chamber for thirty-six hours, showed no signs of solution. The supernatant liquid was clear, and when tested by the biuret reaction gave no indication of the presence of peptone. Mett's tubes were also used, but gave negative results. The same solution, however, tested, as previously described, caused fresh cow's milk to clot firmly in thirty-five to forty-five seconds, and, it may be added, that the particular specimens, which had been allowed to act on the fibrin for thirty-six hours with entirely negative results, also caused prompt curdling of milk in thirty-five to forty-five seconds. These results were obtained constantly under the conditions of experiment described above, and they show conclusively that in a liquid which exhibits both strong peptic and rennetic action the former may be destroyed completely without any apparent change in the latter. Such a result, while not necessarily at variance with the hypothesis of Nencki and Sieber, can scarcely be interpreted otherwise than as demonstrating that the immediate active agent in peptic hydrolysis is distinct from that causing the coagulation of caseinogen. The two enzymes may always occur together in the cell secretion, and they may possibly be united in some such way as that imagined in the hypothesis of Nencki and Sieber, but the effective complex which causes hydrolysis of the protein must be distinct from that acting upon caseinogen.

SUMMARY.

It is shown that in a solution containing both pepsin and rennin the passage of a direct current of ten milliamperes for twenty-five hours results in a complete disappearance of the peptic power, while the action of the rennin is apparently unchanged.

THE BLOOD PRESSURE IN THE ARTERIOLES, CAPILLARIES, AND SMALL VEINS OF THE HUMAN SKIN.

By WARREN PLIMPTON LOMBARD.

[From the Physiological Institute of the University of Würzburg.]

IN the course of experiments on the sense organs of the skin, in the Physiological Institute of the University of Würzburg, it was found that if the skin was wet with glycerine or a transparent oil, one could obtain, with the aid of a microscope, a beautiful picture of the papillae and of the superficial blood vessels.

This was first seen on an area of the skin which had been flooded with white vaseline. An ink fleck on the epithelium looked like red scum floating on the surface of a clear pool, and far above the loops of capillaries, which appeared like scarlet weeds on the yellow bottom of the pool. Each of the capillary loops seemed to spring from a more or less circular little mound. The mounds were papillæ, the tissue of which was so transparent that the red capillaries within stood out strongly from the circles of the bases of the papillæ.

The present methods of measuring the pressure of the blood in the capillaries of the human skin are not satisfactory. They are all based on observations of the change in color which occurs when pressure is applied to the skin. In practice it is difficult to obtain accurate results by such a procedure, whether one observes, like von Kries,[1] the pressure which is just capable of producing a visible pallor, like von Basch,[2] the pressure which causes complete blanching of the skin, or, like von Recklinghausen,[3] the pressure at the instant the skin begins to flush, or when the flushing becomes complete, when a pressure which has driven all the blood out of the superficial vessels is allowed to fall. Von Recklinghausen thought that the pressure at which the flushing became complete was probably that by which all of the capillaries opened up, and was identical with that which was required to close the smallest capillaries under the conditions of the experiment, but that it was in reality about the pressure which normally exists in the very smallest arteries; while the pressure by which the skin first begins to flush, under the conditions, was about the pressure of the next higher arteries. Nevertheless he considered it practical to retain the term "Kapillardruck" for these values (see pages 494 and 493).

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[1] v. Kries: Berichte über die Verhandlungen der königlichen sächsischen Gesellschaft der Wissenschaften zur Leipzig, math.-phys. Cl., 1875, xxvii, p. 147.

[2] v. Basch: Wiener klinische Rundschau, 1900, pp. 549 and 572.

[3] v. Recklinghausen: Archiv für experimentelle Pathologie und Pharmakologie, 1906, lv, p. 463.

The following values, stated in millimetres mercury, were found by these writers for the capillary pressure in the skin of the hand.

VON KRIES.

Averages of many experiments on two normal men.

Distance of finger below crown of head.	Capillary pressure.
0 mm.	24.1 mm. Hg.
205 "	29.2 " "
409 "	37.7 " "
840 "	54.3 " "

VON BASCH.

Experiments on four normal and ninety-eight pathological subjects. Height of hand, not stated. Normal capillary pressure is 25–30 mm. Hg.

VON RECKLINGHAUSEN.

Experiments on himself, the hand being at the level of the upper border of the heart, the day being warm. Averages from experiments at three different times, the first flush being noted: 68, 72.5, 71.3 mm. Average from an experiment in which complete flushing was noted: 55.1 mm.

Not only is it difficult to determine the instant that the color of the skin alters as the external pressure is applied or removed, but the effect of the pressure is at present a matter of conjecture, each of the observers who have given the most attention to this subject having a different view with respect to what change in the color of the skin offers the best criterion of the effects of the external pressure on the capillaries. It seems desirable, therefore, that a method of microscopically observing the effects of external pressure on the superficial vessels of the skin should be developed, the more so because a successful method may prove of clinical value.

METHODS.

Method of observing the blood vessels. — To obtain a view of the most superficial blood vessels of the human skin, it is only necessary to put a drop of glycerine or transparent oil on it, and examine by a strong light with a microscope. One can see the capillaries, even with a hand lens magnifying ten times, but higher powers give better pictures. Although one can use an ordinary microscope, a Zeiss binocular microscope, on account of the large field and working distance which it affords, is of great service. The heavy stand X^b enables the microscope to be given any desired position, and is an invaluable adjunct. Eyepiece No. 2, with objectives No. a1, a2, a3, magnifying ten, twenty-four, and thirty-five times, are the most serviceable. Probably powers much higher than these cannot be used to advantage in experiments to determine the blood pressure. One looks down on the blood vessels, and they and the capillary loops which rise from them towards the surface, do not lie in the same plane. Moreover, when the pressure comes on the skin, it is apt to yield somewhat, and one must, even with a magnification of twenty-four, alter the focus somewhat to ollow the changes which the pressure produces.

In order to apply the pressure vertically to the skin, by direct application of weights, the microscope must be placed a little to one side, and tilted at an angle. The Zeiss stand X^b makes this possible. This tilting of the microscope is an advantage, not only because it puts it out of the way, but because it enables one to look at the capillaries somewhat from the side, and to see the little loops better. The papillæ of the skin also show well from this point of view.

The capillaries are seen by bright daylight, but a Nernst lamp, of the type made by Zeiss for microscopic work, offers some advantages.

It can be fastened by a retort clamp to a stand and given the required position.

The part to be studied must be supported in such a way that it shall be steady, and at the same time be comfortable for the subject, and bring no pressure on the blood vessels or nerves of the arm. An excellent rest for the hand can be readily made of plaster of Paris.

Method of applying the pressure. — Two methods of applying known amounts of pressure to the skin were made use of, and as each of them possessed certain advantages, they will be described in detail.

METHOD A.

This was in principle the same as the "stativ" method employed by von Kries in his experiments, although mechanically it appeared to have some advantages.

A light metal rod carrying at its upper end a little pan, to receive weights, passed through two holes in a guide frame, and was brought in contact with the skin, or with a glass plate resting upon it, by the lowering of the frame. The guide frame was made of a strip of brass, 1½ mm. wide and ⅔ mm. thick, bent to form a square 20 mm. in diameter, and soldered at one corner. A rod fastened to one side of the frame served as a handle. Little holes of the three different sizes, 0.75 mm., 0.65 mm. 0.55 mm., to correspond with the different rods used, were bored just opposite each other in what were to serve as the top and bottom sides of the frame, and to lessen the friction were reamed out, so that there should be almost sharp edges where the rods would come in contact with the frame. The friction experienced by a rod as it slid up and down was very slight. This was tested by letting a rod rest on a scale pan of a fine balance, weighting the other pan to exactly counterbalance, and then watching the oscillations of the balance, while the rod rose and fell in the frame. The rods had various forms. Some were made of small needles, and in order that the weight might be increased, a little pan of very light phosphor-bronze foil was soldered to the eye-end of each needle. Another set of rods, having ends with large surface areas, was made of aluminum wire. The upper end was bent to form a little spiral, the rod projecting from the middle of the spiral, and the spiral itself being horizontal when the rod stood vertically. The spiral gave a surface on which the weights could be placed.

The ends of the rods which were to come in contact with the skin were ground under a microscope to a level surface of known diameter, the surface area being estimated later in fractions of a square millimetre. Thus a variety of rods were constructed, the diameters of the ends being 0.1 mm. to 0.5 mm., the surface areas of the ends $\frac{1}{125}$ mm.^{2} to $\frac{1}{2}$ mm.^{2}, and the weight 0.043 to 0.200 gm.

In order to be able to apply the pressure to still larger surfaces, little plates were cut, by means of a writing diamond, from a cover-glass. The plates were measured under a microscope to within 0.1 mm. Plates having surface areas of 1, 1½, 2, 4, 25 mm.^{2} were used.

In applying a rod to the skin, care was taken to hold it vertical and to avoid lateral pressure. When a rod was used to weight a glass plate resting on the skin, the rod was brought in contact with the middle of the plate, so that the effect of the weight should be evenly distributed over the surface.

Sources of error by Method A. (*a*) *Effect of the size of the surface by which the pressure was applied to the skin.* — It was interesting to observe the effect of pressures applied to the skin by means of rods of the smallest diameters. The smaller the surface pressed upon, the more localized the effect. For example, a rod with an end diameter of 0.1 mm. will, if sufficiently light, when the epithelium is soft and flexible, compress the capillaries of an area of only 0.2 mm. diameter. Unfortunately the writer did not learn how to measure the capillary pressure by the use of such small rods. The values obtained gave absurdly large figures. A rod having an end diameter of 0.1 mm. and weighing 0.043 gm. did not compress all of the capillaries of a given region. The weight seemed small, but estimated for the standard surface, 1 mm.^{2} was really very large in proportion to the size of the surface affected. The end diameter being 0.1 mm., the surface area was $\frac{1}{125}$ mm.^{2} and the pressure exerted was 5375 gm. per mm.^{2}, which is equal to a pressure of 396 mm. mercury, a pressure, for capillaries of medium size, much greater than that of the largest arteries.

In a few other experiments the following values, roughly stated, were obtained for the pressure required to close the most compressible capillaries:

Surface area of rod ¼ mm.², pressure 60–70 mm. Hg.
Surface area of rod ⅓ mm.², pressure 40–50 mm. Hg.
Surface area of rod ½ mm.², pressure 20–30 mm. Hg.
Surface area of rod 1 mm.², pressure 18–20 mm. Hg.

These results were in general in harmony with the observations of von Frey and Kiesow,[4] in their investigations of the conditions which determine the threshold of the pressure points of the skin, when subjected to mechanical excitation. They carefully studied the way in which pressure applied to surfaces of various sizes penetrates and spreads in the underlying tissues, and found that when the surface is below a certain size, about 0.5 mm.², the values of the exciting weights rise very rapidly as the size of the surface decreases.

When surfaces larger than 1 mm.² were used, and the compression of the smallest capillaries was noted, the figures which the writer obtained for the capillary pressure were too small if the capillaries at the borders of the plate were observed, and too large if those under the centre of the plate were watched. For example, when a glass plate 2 mm.², having a surface area of 4 mm.², was used, the value found for the minimal capillary pressure, that is, the pressure required to compress the capillaries which yielded first to the compressing force, was only 12–13 mm.².

When the weight is applied to glass plates larger than 1 mm.², the pressure is not evenly distributed, but is expended largely in stretching the skin at the borders of the plate. That this is the case is shown by the fact that it is at the borders of the plate that the pallor first shows itself and the smallest capillaries are first compressed. The pressure does not bear equally on the whole of the 4 mm.², and the figure for the capillary pressure got by dividing the weight by 4, to reduce the value to the standard surface of 1 mm.², is too small. It is difficult to estimate in such a case just how the pressure is distributed over the surface, and what part of it is employed to overcome the elastic resistance of the skin. Had the weight required to close the smallest capillaries under the middle of the plate been observed, it is probable that too large values would have been obtained.

In one such experiment pressure was brought to bear on the skin by means of a glass plate 5 mm.², a weight being gradually applied to the centre of the plate. As the weight began to press on the plate, the skin at the borders was seen to blanch and the blanching proceeded towards the centre, as the weight pressed more and more. Not even after the full weight had been brought to bear on the plate, were all the capillaries compressed; in the centre of the field many of the capillaries remained apparently unaffected, and throughout most of the field one could see isolated capillary loops which were protected by the irregularities of the skin, and which still contained blood. In this experiment 54.468 gm. acted on a surface of 25 square millimetres, which was equal to a pressure of 160 mm. mercury per square millimetre. The pressure was, however, much less than this at the centre of the field and much more at the border of the plate.

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[4] v. Frey and Kiesow: Zeitschrift für Psychologie, 1899, xx, Fig. 2, p. 146.

When the weight was removed and the glass plate taken off, a deep square pit remained to mark the place to which the pressure had been applied. It was evident that as the weight came on the plate it was forced deeper and deeper into the skin. The skin at the edges of the plate was stretched, and the pressure was largely employed to overcome the elastic resistance of the skin, and only a small amount of the pressure acted on it at the centre of the field. The pit which was left when the plate was removed showed that, as the skin yielded, the lymph, which has less pressure than the blood in the smallest capillaries, was gradually driven out of the lymphatics, the surface of the skin retreating as the lymphatics emptied. That the pitting was not due simply to the compression of the blood vessels was shown by the fact that when the plate was removed, although the blood vessels which had been compressed rapidly filled, the pit remained for a considerable time.

Since the size of the surface to which the pressure is applied largely influences the amount of the weight which is required to compress the capillaries, the question presents itself, just what area of the skin must be subjected to the pressure in order that one may obtain the most reliable values?

The experiments of the writer were too few to permit of a definite answer, but the correspondence between the values got by Method B, and those obtained by Method A when glass plates 1 mm.² were used, was sufficiently close to make it probable that the error which comes from using a surface 1 mm.^{2} would not be great. Certainly much larger plates cannot be used on the back of the hand of the writer, at least, because it is hard to find between the furrows of the skin level areas larger than 1 mm.^{2}. Smaller plates are in practice very undesirable, because of the difficulty of handling them. Finally, it is a decided advantage to use the standard surface, 1 mm.^{2}, and so avoid the necessity of calculations.

(*b*) *The time that the weight is allowed to act.* — This may have an influence on the amount of pressure required to compress the capillaries. As has just been said, when one applies the pressure by means of plates larger than 1 mm.^{2}, the pressure acts at first chiefly at the borders of the plate in overcoming the elastic tension of the skin. As the skin gradually yields under the weight, the lymph is driven out, and the plate sinks deeper into the skin, the effect of the pressure progressing from the edges towards the centre of the field. When plates not larger than 1 mm.^{2} are employed, however, the effects of the pressure are seen quite promptly, and the time factor appears to be of much less importance.

(*c*) *Influence of the condition of the skin.* — The amount of the pressure absorbed in overcoming the resistance of the epithelium depends to a considerable extent on the condition of the epithelium at the time. When the skin is dry, the epithelium is stiff and horny, and acts like a firm crust to oppose the effects of the pressure. One of the little areas bounded by the furrows has a convex surface, which acts like an arch to support the pressure brought on it. As the pressure increases, the sides of the arch bend out, the crown is depressed, and the capillaries in the centre of the area feel the pressure sooner than those at the borders. That the skin offers much more resistance when it is dry than when it is moist and flexible, was first observed in experiments in which the threshold of the pressure points was studied. It was found that moistening the skin with vaseline lowered the threshold markedly. The same thing was seen when the pressure required to close the capillaries was sought. The following experiment, which was only one of a number giving similar results, is an illustration.

Experiment, March 22, 1911, on the radial side of the back of the left hand of W. P. L.

No oil had been applied to the skin for three days. It looked dry and slightly chapped. Under a microscope one could see that little flakes of epithelium were becoming detached. The surface epithelium was evidently horny, and the irregularities of the surface appeared to be much more than when the skin is moist. There were but few areas of as much as 1 mm.^{2} to be found, and none of these had an absolutely level surface. In most cases the surface was somewhat convex, and in the areas less than 1 mm.^{2} the convexity was marked, the skin arching up from furrows 1/20–1/10 mm. deep. The hand was 13 cm..below the second, left intercostal space, and 39 cm. below the crown of the head. The pressure was exerted by weights applied to the centre of a plate 1 mm.^{2}, by a rod held in the guide frame. The plate exactly fitted the area chosen for the experiment, without overlapping the furrows. In the tests the minimal capillary pressure was sought, *i. e.*, the pressure of the blood in the most compressible capillaries.

TEST I.[1]

Time.	Weight.	Pressure.	Observed effect.
	gm.	mm. Hg.	
3.00	....	....	Vaseline put on skin and plate adjusted.
3.10	0.307	22.6	Partly compressed capillaries.
...	0.307	22.6	" " "
...	0.317	23.3	Closed smallest capillaries.
...	0.312	22.9	" " "
...	0.309	22.7	" " "
...	0.307	22.6	Closed smallest capillaries only if the weight was allowed to act for a long time.

[1] The estimated pressure was 22.7 mm. mercury.

In the experiment the pressure required to close the smallest capillaries fell from 22.7 to 18.5 mm. mercury as a result of the increased flexibility of the skin produced by the vaseline. The result was not due to the fact that Tests I, II, and IV were made on the same area, because the plate was removed between the tests, and sufficient time elapsed between them to allow the skin to recover. Test III, which was made on a neighboring area of dry skin, gave about the same result as Test I.

There appears to be no accurate way of estimating the varying resistance of the skin, and so one must apply the pressure in such a way as to call out the resistance of the skin as little as possible. To do this, one should make the skin soft and flexible, and if the pressure is to be applied by means of a plate, should avoid using plates larger than 1 mm.[2].

TEST II.[1]

THE SAME AREA AS IN TEST I.

Time.	Weight.	Pressure.	Observed effect.
	gm.	Mm. Hg.	
4.30	0.257	18.9	Did not compress capillaries.
. . .	0.267	19.6	" " " "
. . .	0.277	20.4	Closed smallest capillaries.
. . .	0.272	20.0	Did not compress capillaries.
. . .	0.273	20.1	Closed smallest capillaries.

[1] The estimated pressure was 20.1 mm. mercury.

(*d*) *What part of the field should be watched in observing the effect of the pressure?* — The fact that the pressure is not distributed equally over the whole of the surface on which the plate rests, may introduce a considerable error. Even with a plate only 1 mm.[2], the pressure tends to act most at the borders of the plate, and for this reason it is probably best not to watch the compression of the capillaries at this part of the field. It frequently happened, however, when the skin was dry that the capillaries towards the middle of the field were most affected. If there are any irregularities of the surface, the most elevated portions are, of course, subjected to the pressure. Evidently the effects of the pressure in a number of different parts of the field must be observed, if one would avoid the errors which may come from these causes.

(*e*) *By how much must the weights differ to produce recognizable effects on the capillaries?* — Von Kries employed in his experiments plates 2, 5, 4, 25 mm.^{2}. He found that smaller plates could not be used, because of the difficulty of observing a change in the color of the skin. The use of the microscope largely does away with this difficulty. Von Kries also found that it was not practical to employ weights differing less than 0.25 gm. The influence of smaller weights on the color was too slight to be detected with certainty.

TEST III.[1]

ANOTHER AREA, WHERE THE SKIN WAS DRY.

Time.	Weight.	Pressure.	Observed effect.
	gm.	mm. Hg.	
5.30	0.307	22.6	Did not compress capillaries.
...	0.312	22.9	Closed smallest capillaries.
...	0.310	22.8	" " "

[1] The estimated pressure was 22.8 mm. mercury.

When one uses a plate 1 mm.^{2}, and observes under a microscope the effect of the pressure on the separate capillaries, it frequently happens that the effects of weights differing 0.002 or even 0.001 gm. can be observed; — see experiment of March 22, page 342. Where the epidermis is thicker and dryer, and the total weight which has to be used is larger, it may be necessary to employ weights differing as much as 0.005 gm. The error from this source would therefore be 0.15–0.37 mm. mercury.

METHOD B.

The apparatus employed to compress the capillaries by this method resembles in many respects that used by Roy and Graham Brown,[5] and later by Lapinsky,[6] in their study of the capillaries of the frog, and in other respects that used by von Recklinghausen[7] and von Basch[8] in their study of the pressures of the capillaries. The apparatus of the writer (see Fig. 1) consisted of a brass cone, having a little pressure chamber at its base, the roof of the chamber being a thin cover-glass, the bottom a thin membrane having a little opening in the centre. The cone was 22 mm. high, the outside diameter of the top 35 mm., the diameter of the bottom 24 mm., and the thickness of the wall at the top and bottom 3 mm. The top and bottom surfaces of the wall were flat. The total weight was about 70 gm. The bright metal wall of the cone reflected the light down to the bottom, and the weight helped to hold it in position on the skin. The pressure chamber at the bottom of the cone had an inside diameter of 18 mm., and a depth of 3½ mm. The cover-glass forming the roof was fastened in air-tight with sealing wax. The membrane forming the bottom was spread loosely over the surface, and was turned up over the edge where it was tied by a thread into

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[5] ROY and GRAHAM BROWN: Journal of physiology, 1879–80, ii, p. 325.

[6] LAPINSKY: Archiv für Physiologie, Suppl., 1899, pp. 477–509.

[7] v. RECKLINGHAUSEN: *Loc. cit.*, p. 468.

[8] v. BASCH: *Loc. cit.*

TEST IV.[1]

SAME AREA AS IN TESTS I AND II.

Time.	Weight.	Pressure.	Observed effect.
	gm.	mm. Hg.	
6.00	0.307	22.6	Closed smallest capillaries.
...	0.297	21.8	" " "
...	0.287	21.1	" " "
...	0.277	20.4	" " "
...	0.267	19.6	" " "
...	0,257	18.9	" " "
...	0.207	15.2	Did not compress capillaries.
...	0.227	16.7	" " " "
...	0.237	17.4	" " " "
...	0.247	18.2	" " " "
...	0.257	18.9	Closed smallest capillaries.
...	0.252	18.5	" " "

[1] The estimated pressure was 18.5 mm. mercury.

a groove. The membrane was "gold-beaters' skin." When it was applied, a circular hole was cut in the centre of the piece with a 5 mm. cork-borer, and then the membrane was thoroughly wet in water, and laid loosely over the bottom of the cone and tied into the groove. A hole bored diagonally downward through the side of the cone, near the bottom, opened into the pressure chamber as near to the top as was possible and yet allow room for the cover-glass forming the roof. Into the outer end of the hole a small brass tube 20 mm. long, with an inside diameter of 3 mm., was soldered. This tube connected by a very short piece of rubber tubing with a piece of glass tubing, 10 mm. diameter and 35 mm. long, which had been drawn at each end and given a shoulder. A reservoir was thus formed, in which there could be a store of the glycerine with which the pressure chamber was filled when in use. To the upper end of the reservoir a piece of rubber tubing was fastened, and the free end of this tube was closed, when desired, by a little glass plug. The reservoir connected with one of the side openings of a Wolf-bottle, serving as expansion chamber for the air employed to produce the pressure. The connection was made by a cannula, fastened to the tube coming from the Wolf-bottle, and drawn to fit the rubber tube on the reservoir. It was not necessary to fasten a ligature at this place. In the middle of the Wolf-bottle was a three-way stopcock, the bottom opening of which communicated with the bottle, the top opening with the outside air, and the side opening with a manometer. The other opening of the bottle connected with the cautery bulb to compress the air. The manometer was a

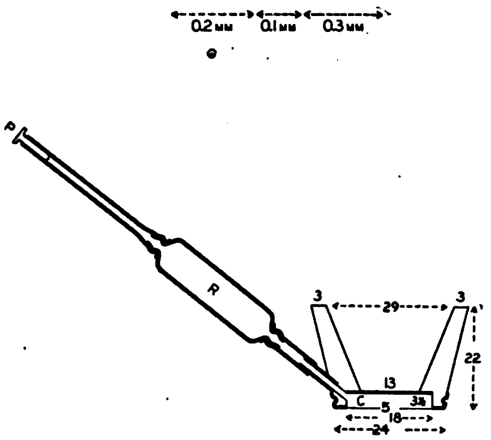

FIGURE 1. — Pressure chamber of Method B. *C*, the pressure chamber, with cover-glass roof and membrane floor; *R*, reservoir for glycerine; *P*, glass plug at place where tube connects with a Wolf-bottle, and through this, with a bulb for raising the pressure, the manometer, and the opening to the outside air. The measurements are stated in millimetres.

mercury manometer, and consisted of the ordinary U tube, fastened to a stand and so placed that the millimetre scale could be easily read by the observer.

When the air pressure was applied, the weight of the cone was not sufficient to prevent the glycerine from being forced out beneath it. It was therefore necessary to press the cone down upon the skin, and this was done by means of a ring fastened to a rod which was clamped to a stand.

The base of the cone where it rested on the skin was sufficiently wide, so that its pressure did not affect the blood vessels under the opening in the centre of the membrane. The superficial vessels are supplied by richly anastomosing plexuses of arteries and veins lying beneath them (Spalteholz [9]), and pressure on neighboring parts has little effect.

The only difficulty encountered in filling the pressure chamber with glycerine was the getting rid of the air. The following method proved effective. The shorter tube on the reservoir was connected with the tube coming from the pressure chamber, then the cone was turned, membrane side down, and the membrane dipped into glycerine in a shallow dish, the opening in the membrane being kept well under the glycerine, and the cone being moved up and down against the bottom of the dish, so that the pumping motion should fill the pressure chamber and the reservoir. Tilting the dish slightly, so that the tube coming from the pressure chamber should be higher than the rest of the chamber, helped to expel the air. As soon as the reservoir was filled and the air was out of the pressure chamber, the longer tube of the reservoir was closed by the little glass plug. The cone was then turned upside down, and enough glycerine was put with a dropper through the hole in the membrane to fill the pressure chamber to overflowing. A cover-glass was then slid over the opening.

To apply the cone to the skin, it was turned over, put on a part of the skin close to the place to be studied, and slid along the skin so that the cover-glass should be rubbed out from under it. When the cone was to be removed from the skin, the longer tube connected with the reservoir was detached and the glass plug was inserted.

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[9] SPALTEHOLZ: Archiv für Anatomie, 1893, p. 1. Handatlas der Anatomie der Menschen, 1903, iii, p. 840.

Sources of error by Method B. — Undoubtedly by this method, just as by Method A, a portion of the pressure is employed in overcoming the resistance of the epithelium and the connective tissue of the corium, the amount of pressure thus absorbed being greater when the skin is dry. Moreover, the effect of the pressure appeared to be felt most at the border of the field, close to the edge of the membrane.

The influence of the time that the pressure was applied showed markedly by this method, just as it did by Method A when large surfaces were subjected to pressure. For example, in the test at 6.30 P. M., March 29, the pressure was rapidly raised to 140 mm. mercury. For a short time a few of the largest vessels were seen, but as the pressure continued, they disappeared. The pressure was then gradually lowered. Three and a quarter minutes later, the pressure had fallen to 60 mm., but the field was still white. As the pressure fell during the next one and a quarter minutes to 50 mm., the larger vessels and a few of the capillary loops appeared and the background of the field began to flush.

It was not ascertained how sensitive this method could be made. Apparently the method has many practical advantages over Method A, although it appears to be less delicate.

RESULTS.

The appearance and nature of the superficial vessels of the human skin. — If a drop of glycerine be placed on the skin of the back of the hand, and it be examined under a microscope magnifying twenty-four times, the epithelium is not seen, and an unobstructed view of the underlying corium with its papillæ is obtained. Moreover, a strong light will penetrate a short distance into the corium itself, and its connective tissue is sufficiently transparent to permit of a view of the capillaries in the papillæ, and even of the more superficial of the small vessels of the subpapillary layer. The picture obtained depends on the condition of the skin at the time. When the vessels are contracted, one sees only a few capillary loops, with here and there a vessel faintly outlined against a pale yellow background. When the vessels are full of blood, the background has a rose tint, the red being unevenly distributed, and often occurring in irregular, somewhat elongated patches, toward the edges of which the rose color fades into light yellow. In these patches one can often see the outlines of small vessels which evidently belong to the subpapillary plexus, those which lie deepest in the tissue showing like indistinct red shadows, suggesting pink coral beneath a yellow veil. When one of these vessels comes closer to the surface, its small branches and the tiny twigs which become the capillary loops can be seen distinctly. I have just been looking at such a picture, and was able to count sixteen loops springing from one of these superficial vessels, five of the loops coming off at regular intervals from its terminal branch, and the rest from other little branches. The loops differ in length, the spread of the arc, and the calibre of the vessels of which they are composed. Sometimes the loops are simple arcs, but very often they are twisted on themselves. Not infrequently one limb of the loop, just as in the case just cited, can be seen to come off from a branch of a small vessel, but I have thus far failed to trace the other limb of a loop to the other vessel with which it must connect. The apparently free limb descends from the loop, often winding about the ascending limb, grows more and more indistinct, and fades from sight in the tissue of the corium.

The question arises, what is the nature of these vessels with which the loops are seen to connect? To judge from the way they have been seen to react to external pressure, they are in some cases the branches of the smallest veins, and in others the branches of arterioles, for in some cases, as the external pressure was gradually increased, these little superficial vessels faded from sight before the capillary loops, and in other cases they outlasted the capillary loops.

A much more striking picture of the capillary loops is obtained in the skin at the base of the thumb nail, or of one of the finger nails, because here the loops may be seen in profile, while in most parts of the skin one obtains only a partial side view.

The skin where it laps over the thumb nail of the left hand of the writer is, for a distance of three tenths of a millimetre from the edge, dry and horny looking. Just proximal to this, the epidermis when examined under glycerine is transparent, and the base of the field has a light rose-colored flush. Five tenths of a millimetre from the horny band, small vessels may be seen coming out from the deeper layers of the corium, and these vessels give off branches which run more or less parallel to the edge of the skin.

The outlines of the other, more centrally lying vessels, which apparently anastamose with these and with each other, show indistinctly through the transparent connective tissue of the corium. The faintly seen anastomosing plexus of vessels is probably the subpapillary venous plexus described by the histologists, and the more distal vessels which communicate with them, and come closer to the surface, probably represent the most distal border of that plexus. For convenience we will refer to these vessels and their larger branches as the border plexus. The branches, as has been said, run about parallel with the edge of the skin and are joined together by small terminal branches. That such a communication exists was proved by the following experiment.

A black glass rod which had been drawn out to fine point was pressed lightly on the skin over the course of one of these vessels, and the blood was seen to leave the vessel in both directions. The rod was then slid along the vessel, and the blood was forced along in front of the rod, at the same time the vessel filled again behind the rod; also when the rod was slid back in the opposite direction along the vessel, the same movements of the blood were seen. The change of color came quickly, and the experiment showed that the anastamoses were free, and that there were no valves in these most superficial veins. Spalteholz[10] thought to have observed valves in some of the veins forming the four venous plexuses which he found in the skin.

From the border plexus very fine branches are seen to be given off, both centrally and distally. These branches are probably analogous to the fine branches which are often seen in the skin of the back of the hand, coming close to the surface of the corium. The central branches receive the blood from the short capillary loops which rise up more or less vertically towards the surface of the corium, while the distal branches receive blood from the long capillary loops which run horizontally toward the edge of the skin where it laps over the nail.

The loops which extend distally are very long, and in some cases exceedingly delicate reddish threads which have a wavy and often twisted course. The projected picture gives a length of about 0.2 mm., and such a loop ends, or rather the vessel turns back on itself, about

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[10] SPALTEHOLZ: Archiv für Anatomie, 1893, p. 48.

0.1 mm. from the horny skin bordering the picture. The place where the loops spring from the branches of the main vessel can be made out by focusing in some cases, distinctly, but the other ends of the loops cannot be traced to any vessels.

It was at first supposed that the main vessels were arteries, and a useless search for the corresponding veins was made. Fortunately there was one loop which was much larger or much more injected than the rest. It stood out strongly and distinctly red, and as it was all in nearly the same plane, it could be followed in its details from where it left a branch of the main vessel to where it seemed to come to an abrupt end. Certainly it did not end in any visible vessel. This loop, like the others, had a wavy, twisted course, and a sketch was made (see Fig. 2).

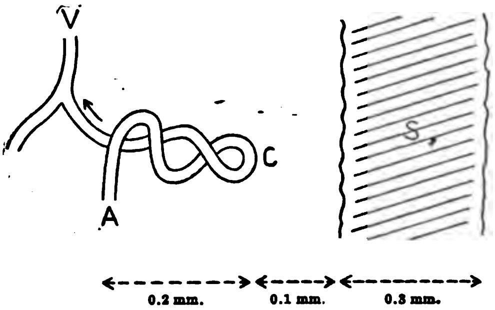

FIGURE 2. — A capillary loop in the skin at the base of the thumb nail. *A*, limb of capillary leading to an arteriole; *C*, capillary loop; *V*, branch of vein; *S*, dry skin at the base of nail.

One could not determine, from looking at it, which limb of the loop crossed over or under the other, but this was definitely brought out by light pressure applied to the skin just distal to the loop. As the pressure was increased, the blood was driven out of the loop, but the loop did not empty all at once; the most distal part of the loop first lost its blood, and then the blood was driven gradually out of one side of the loop in the direction of the arrow and towards the main vessel. At this time one could see the whole course of the rest of the loop, and see which part of it lay over, and which part under, the portion of the loop first yielding to the pressure. As the pressure was increased the most resisting part of the loop lost its blood, the blood being driven back towards what appeared to be the end of the loop. The whole condition was now clear; the main vessels, which at first were supposed to be arteries, were veins; the arteries lay deeper down in the corium, and could not be seen; the apparent end of the loop was really the place where it came out from the depths of the corium. Evidently it is here true of these vessels, as it is of the larger vessels of the skin, that the veins are more superficial and the arteries lie deeper and are better protected. In the skin of the back of the hand, however, branches of the arterioles as well as of the smallest veins may come close to the surface of the corium, and may be seen to give off capillary loops. This is shown by the fact that one may see there vessels which yield to pressure before the capillaries and others which resist a pressure which drives the blood out of all the capillary loops.

TABLE I.

EXPERIMENTS ON THE BACK OF THE LEFT HAND OF THE WRITER, THE HAND BEING 100 MM. BELOW THE SECOND INTERCOSTAL SPACE.

Date.	Time.	Test.	Condition of the skin.	Pressure.
Mar.				mm. Hg.
20	...	I	The skin was moderately soft.	19.2
22	3.10	I	The skin was dry.	22.7
..	4.30	II	Same place as in I; skin was softer.	20.1
..	5.30	III	Neighboring area; skin was dry.	22.1
..	6.00	IV	Same place as in II, skin was still softer.	18.5
23	6.10	I, *a*	Skin was moderately soft.	18.8
..	...	I, *b*	Circulation changed during test.	22.6
..	6.25	II	Skin was softer.	16.8
..	7.15	III	Skin was still softer.	15.1
26	4.45	I	Skin was moderately soft.	19.1

The capillary pressures observed by Method A. — Most of the experiments made by this method were directed to studying the method itself, and comparatively few tests were made by applying the external pressure to a surface 1 mm.^{2}, which appeared to give the most reliable results. The pressures observed *for the most compressible capillaries* are given in Tables I and II.

In the experiments on the back of the hand of the writer by Method A, the average pressure in the most compressible capillaries was 19.5 mm. mercury. In the one experiment on the skin at the base of a finger nail of the writer made by Method B, the pressure was

20 mm. The average of the observations made by Method A on the skin at the base of a nail, in the case of the woman and boy who acted as subjects, was 21 mm. In the case of the man, however, the pressure was 27.6 mm.; possibly this may be accounted for by the fact that in this case the room temperature was much lower than it was when the others were examined. H. and B. Ballentine,[11] using von Kries' method, found that cold raises the capillary pressure. Although the total number of observations was small, probably 20 mm. mercury is a fair estimate of the pressure in the most compressible capillaries

TABLE II.

EXPERIMENTS ON THE SKIN AT THE BASE OF A FINGER NAIL OF THE LEFT HAND. THE HEIGHT OF THE HAND WITH RESPECT TO THE UPPER BORDER OF THE HEART, ESTIMATED FROM THE SECOND INTERCOSTAL SPACE IN MILLIMETRES.

Sex.	Age.	Height of hand.	Time.	Remarks.	Pressure.
					mm. Hg.
Woman	50 +	− 30	5.00 P. M.	Skin was moderately soft.	21.1
			6.00 P. M.	......	22.0
			6.30 P. M.	Effect of thunder.	21.6–21.1
Man	50 +	− 80	9.42 A. M.	Hand cold. Room temperature 14½ R.	27.6
Boy	12	+ 10	2.46 P. M. 4.10 P. M.	Hand chapped and skin dry. Between 2.46 and 4.10 the subject took a light lunch.	23.5 17.1 19.8
....	....	....	5.00 P. M.	......	21.3

TABLE III.

ONE EXPERIMENT WAS MADE BY METHOD B, ON THE SKIN AT THE ROOT OF A FINGER NAIL ON THE LEFT HAND OF THE WRITER.

Sex.	Age.	Height of hand.	Time.	Remarks.	Pressure.
					mm. Hg.
Man	56	− 100	7.00 P. M.	Skin was moderately dry.	20

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[11] H. and B. BALLENTINE: Journal of the Boston Society of Medical Sciences, 1898–1899, iii, p. 330.

of the skin of the back of the hand and at the base of a finger nail under ordinary conditions.

The pressure required to close capillaries of medium size was considerably higher, and the most resisting capillaries withstood nearly as high a pressure as the branches of the arterioles. The most superficial of the small veins began to be compressed somewhat earlier than the smallest capillaries, and the veins of the subpapillary venous plexus still earlier. No definite figures for these pressures were obtained by Method A.

TABLE IV.

Weight. gm.	Pressure. mm. Hg.	Remarks.
0.230	21.1	Did not compress the capillaries.
0.232	21.25	Did not compress the capillaries.
0.235	21.5	Did not compress the capillaries.
0.240	21.84	Closed the smallest capillaries.
0.237	21.6	Closed the smallest capillaries. The skin looked a little more flushed than it had, and pressure caused a slight pallor of the background of the field. The subject said she felt like sneezing.
0.237	21.6	Closed some very small capillaries. Just after this observation there was a severe thunder clap, and some of the smallest capillaries which had before been in sight could not be seen.
0.230	21.1	Closed some small capillaries. This was soon after followed by another thunderclap, and again some of the smallest capillaries disappeared, but soon reappeared.
0.220	20.4	Did not compress the capillaries.
0.230	21.1	Did not compress the capillaries.

One of the tests reported in Table II was interesting, because a slight change in the capillary pressure was caused by a thunder storm which was in progress. This was the test at 6.30 P. M., the last of the three, which was made on the skin at the base of the nail of the third finger of the woman. The subject sat at the opposite side of the table from the observer, the arm resting on a cushion, and the finger being steadied by passing through a loosely fitting brass tube, soldered to the end of a rod, which was clamped to a standard. The end of the finger rested on a pad of cotton. The finger was 34 cm. below the crown of the head and 3 cm. below the upper border of the heart, estimated from the second intercostal space. The pressures observed in this test are given in Table IV.

The capillary pressure was estimated to be 21.6 mm. mercury. As a result of the thunder, the pressure was temporarily decreased, becoming 21.1 mm. or less, probably because of the constriction of the small arteries.

TABLE V.

No.	Distance of hand below crown of head.	Pressure.	Subject.	Observer.
	mm.	mm. Hg.		
1	0	24.1	Man	von Kries
2	205	29.2	Man	von Kries
3	300	17.1–23.5	Boy	W. P. L.
4	340	21.1–21.6	Woman	W. P. L.
5	430	27.6	Man	W. P. L.
6	490	37.7	Man	von Kries
7	840	54.3	Man	von Kries

In Table V the three experiments made on the skin at the base of the finger nail, on three different subjects, by the modified von Kries method, Method A, are compared with the average results reported by von Kries.

The pressures observed by the writer were somewhat less than those reported by von Kries, which is easily accounted for, since the writer employed a plate somewhat smaller than those used by von Kries, and one can more readily observe the closure of the smallest capillaries by the use of a microscope, than he can detect a change in the color of the skin by the unaided eye.

The pressures observed by Method B. — Three experiments were made by this method on the radial side of the back of the left hand, and one at the base of the thumb nail of the writer. The last has been already referred to (see Table III), page 354.

In the experiments on the skin of the back of the hand, there were seen at the start a few delicate arterioles, their branches, and the capillary loops arising from them; other capillary loops which connected with veinlets or their branches; and the reddish yellow background of the corium, in which the faint outlines of deeper-lying vessels were visible. When gradually increasing pressure was applied, the background began to pale before the more superficial vessels appeared to be affected. These vessels lost their blood in the following order: some of the small superficial veins, the smallest capillaries, more of the small veins, more of the capillaries, finally, after all the veins had been compressed, the last of the capillaries and the branches of the arterioles and the arterioles themselves. The background, as a whole, became more and more pale as the pressure increased, until finally the whole field was white (see Table VI).

When the pressure was lowered, the blood tended to return to the small vessels in the reverse order, but the rate at which the pressure fell influenced the process. If the pressure was lowered slowly, the corium began to redden when only a very few of the capillary loops had begun to show; if it was lowered quickly, a considerable number of loops appeared before any very evident change in the color of the corium was noted, although the filling of the veins and the flushing of the background rapidly followed. If the pressure was raised enough to drive all of the blood out of the surface of the skin, and the pressure was maintained for several minutes, the lowering of the pressure was followed by hyperæmia. The increased injection of the vessels caused the capillary loops to look like bits of brilliant red coral, instead of pink, as they did before the pressure was raised, and the background of the field became much redder than it had been before the pressure was applied.

The hand was about 10 cm. below the upper border of the heart, and 42 cm. below the crown of the head. In these experiments the pressure was raised and lowered at various rates, and in general was not allowed to act longer than was required to observe the effect. In each experiment a number of tests was made, and between the tests time was allowed for the vessels to recover from the effects of the preceding compression. Attention has already been drawn to the fact that the full effect of the pressure was not seen at once, but that its action on the vessels increased as it overcame the resistance of the skin. During each test the eye was fixed on one particular group of capillary loops and vessels, and the effect of the pressure was noted. The results of two of the experiments have been brought together in the following table; those of the other day, March 29, although not in conflict, have been omitted because of a complication which entered into the experiment, and which will be referred to shortly. The external pressure is stated in millimetres mercury.

TABLE VI.

Back of hand, Mar. 28.	Base of thumb nail, Mar. 30.	Observed effect.
..	15	Background began to show pallor.
..	20	Smallest superficial veins began to pale.
20	20	Smallest capillaries disappeared.
..	25	Many of the superficial veins had disappeared.
35	30	Background showed considerable pallor.
40	40	Most of the superficial veins had disappeared, but many capillary loops still showed.
45	..	Background was decidedly pale.
50	50	Most of the capillary loops had disappeared.
60	60	A very few capillary loops were still to be seen.
..	65	The last of the veins had disappeared.
70	75	All the capillary loops had disappeared.
75	..	One arteriole still showed.

In these experiments the background was affected by the pressure before the more superficial vessels; the small superficial veins yielded to the pressure slightly before the capillary loops emptying into them; the smallest capillaries were closed at a pressure of 20 mm. mercury; the medium capillaries were closed at 35–45 mm. mercury; a few of the largest capillaries were still to be seen at 60 mm. but had disappeared at 70 mm.; and one arteriole resisted a pressure of 75 mm.

Vaso-dilatation caused by pressure on a nerve. — In the course of the other experiments made by Method B on the skin of the back of the hand of the writer, March 27, an effect of pressure on the nerves of the forearm was observed. (See Table VII.)

Although this was an isolated observation, it is recorded because of its suggestiveness. There can be no doubt but that one should use great care to avoid pressure on the nerves of the arm when the circulation in the part is to be studied.

TABLE VII.

Time.	Pressure.	Observations.
	mm. Hg.	
5.30 P. M.	26	Some of the smallest capillary loops were closed. Just after this it was noticed that the hand was becoming numb, because of the pressure of the forearm on the supporting block. It was also noticed that the capillary loops and the veins of the subpapillary plexus looked larger, had a more brilliant color, and so showed more distinctly than at the beginning of the experiment.
6.10 P. M.	25	A small capillary loop was seen to close. A cushion had been placed under the forearm and the numbness had passed off, but the vessels still showed with unusual distinctness.
6.18 P. M.	15	A number of small capillary loops disappeared.
	18	At another part of the field the smallest loops were closed.

Is the pressure the same in all of the capillaries of a small area of the skin, for example 1 mm. square? — The capillary pressure is often referred to, as if it was the same in all of the capillaries of a given part of the body. Probably this has been due to the difficulty of estimating the pressure in the different capillaries. Roy and Graham Brown[12] thought that their experiments on the web of the foot of the frog proved that these capillaries are not only of different sizes, but that they are capable of active contraction, and that they may change their size independently of each other. A cursory examination of the capillary loops of the human skin shows them to differ greatly in size and length. Even those which are seen to come off as twigs from the same little vessel differ, and must offer different resistances to the passage of the blood, and themselves have different pressures. This is best seen in the capillaries at the base of a finger nail. Whether the relative size of the neighboring capillaries actively changes from time to time, was not determined.

When gradually increasing pressure is applied to the skin, the capillaries do not all yield at the same time. The mechanical conditions which determine the way in which pressure applied to the surface spreads through the underlying tissues, do not suffice to account for the differences observed. It is not the capillaries which are nearest to the surface which always yield first. Capillary loops which are close together, and which must have been subjected to nearly the same pressures, require different degrees of pressure for their compression. For example, in one of the experiments made by Method A, the following note was made: "A number of very fine capillary loops disappear at a pressure of 15 mm. mercury, while others from the same arterial branch continue to show well. At another part of the field, some of the loops disappear at a pressure of 18 mm., while neighboring loops are apparently unaffected." Von Recklinghausen, who considers the pressure to be about the same in all the capillaries of a district, explains the fact that they are closed by different amounts of pressure, on the ground that, as part of the capillaries are compressed, the blood pressure in the other loops receiving the same arterial supply would be raised, and consequently they would require greater pressures for their compression. There can be no doubt but that this would be the case, and would be more and more effective the higher the pressure was raised, but it does not seem as if it would suffice to account for the apparent differences in capillary pressures observed, when the external pressure was gradually raised and when relatively few of the capillaries had been closed.

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[12] Roy and Graham Brown: *Loc. cit.*, p. 333.

Moreover, if the external pressure be raised until all of the blood be driven out of the superficial vessels of the skin, and then be suddenly lowered, all of the capillaries should fill at the same time if they offered equal resistances to the pressure; but this does not happen; those which are the last to close, when the blood is being driven out by the rising pressure, are the first to open as the pressure falls.

One can roughly divide the capillaries into three classes: the small capillaries, which yield to very small pressures, 15 to 20 mm. mercury; the medium capillaries, which are compressed by 35 to 45 mm., and this is by far the largest number; and the largest capillaries, which resist pressures almost as high as the smallest branches of the arterioles, perhaps 60 to 70 mm. These figures would of course vary with the condition of the circulation at the time. Moreover, such a classification is purely arbitrary, as probably every degree of pressure exists in the capillaries of a part, from pressures but little above those of the venous rootlets to pressures but little less than those in the smallest branches of the arterioles.

The figure which one obtains for the minimal capillary pressure, *i. e.*, that required to compress the most compressible capillaries, seems to be fairly reliable, although, because of the resistance of the tissues of the skin, it is a little too high. The pressures which are observed to compress the capillaries offering a medium resistance, are probably still higher than the normal pressures in these vessels, because the greater the number of capillaries closed the greater must be the pressure in those which remain open. Finally, the pressures which are needed to compress the most resisting capillaries are very much higher than the normal pressures in these vessels, and even higher than those which normally rule in the branches of the arterioles from which they spring.

Apparently there is a fall in the pressure of the blood in its passage from the small arteries, through the capillaries to the subpapillary venous plexus, of 40 to 50 mm. mercury.

CONCLUSIONS.

If a drop of glycerine or of a transparent oil be placed on the human skin, and it be examined under a microscope, by a strong light, a remarkably fine picture of the superficial blood vessels may be obtained.

The pressure of the blood can be ascertained with considerable accuracy, by applying pressure to the skin by either of the two methods described in this paper, and observing the effects of the pressure under a microscope. The estimates of the pressure of the blood in the smallest veins and in the most compressible capillary loops is probably more reliable than that of the blood in the larger capillaries and arterioles.

Experiments were made on the skin of the back of the hand and at the base of the finger nail. When the hand was about 10 cm. below the upper border of the heart, the temperature was about 20 C., and the conditions were normal, the vessels were seen to be compressed by the following pressures, stated in millimetres mercury.

Subpapillary venous plexus	10–15
Most superficial and smallest veins	15–20
Most compressible capillaries	15–25
Average capillaries	35–45
Most resisting capillaries and arterioles	60–70

Although the total number of experiments was small, these figures probably give a fair picture of the way the pressure of the blood falls in its passage from the arterioles, through the capillaries, to the small veins.

Incidentally, the effect of the condition of the skin, of a meal, of psychic activity, of pressure on a nerve, of cold, and of hyperæmia following prolonged pressure on the skin, were observed.

THE STHENIC EFFECT OF EPINEPHRIN UPON INTESTINE.

BY R. G. HOSKINS.

[From the Laboratory of Physiology in the Harvard Medical School.]

THE effects of epinephrin upon the intestine have been differently reported by different observers. Ott[1] seems to have been the first to note any definite influence. He stated in 1897 that adrenal extract relaxes the intestine; he observed that it also increases peristalsis, but in subsequent publications has made no further mention of this fact. Bunch[2] reported, as a result of intravenous injection of 0.32 gm. of adrenal extract, an increased tonus of the gut. Magnus,[3] working with isolated segments of intestine, saw a single instance in which increased peristalsis occurred following the use of suprarenin, but regarded it as unique. Boruttau,[4] Pal,[5] Langley,[6] Magnus,[7] Elliott,[8] and Cannon and de la Paz[9] have observed inhibition of intestinal activity as the characteristic effect.

During the course of a recent investigation of various biologic tests for epinephrin, a quantitative study has been made of the effects of adrenalin in various dilutions upon isolated segments of rabbit's intestine.

The technique employed was similar to that of other investigators in this field.[10] Pieces of small intestine 3 or 4 cm. long were removed

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[1] OTT: Medical bulletin, 1897, xix, p. 376.
[2] BUNCH: Journal of physiology, 1898, xxii, p. 366.
[3] MAGNUS: Archiv für die gesammte Physiologie, 1905, cviii, p. 50.
[4] BORUTTAU: *Ibid.*, 1899, lxxviii, p. 97.
[5] PAL: Wiener medizinische Presse, 1901, xlii, p. 2023.
[6] LANGLEY: Journal of physiology, 1901, xxvii, p. 249.
[7] MAGNUS: *Loc. cit.*, p. 48.
[8] ELLIOTT: Journal of physiology, 1905, xxxii, pp. 401–467.
[9] CANNON and DE LA PAZ: This journal, 1911, xxviii, p. 64.
[10] FRÄNKEL: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lx, p. 395.

from the animal to warm oxygenated Ringer's solution. To permit free access of oxygen they were suspended from glass hooks. Almost immediately such strips begin rhythmic contractions and continue in activity for hours. From time to time, as required, segments were used to obtain graphic records. They were attached to a writing lever and suspended in a small glass cylinder into which oxygen was constantly bubbling through a supply tube connecting with the base. The lower end of the segment was held by a serre-fine attached to a thread leading out through the supply tube. The cylinder was kept constantly surrounded by water at 37°. A second small tube connecting with the base and provided with a short segment of rubber tube at its free end permitted changing the fluids in the cylinder with a minimal disturbance of the tissues. This was done by inserting a pipette into the rubber tube and aspirating off the fluid. In such experiments, as Fränkel has noted, the animal from which the strips are taken should not be etherized, since ether materially diminishes peristalsis. The animals used in this research were killed by a blow on the back of the neck or anæsthetized with urethane, which does not have the disturbing effect of ether.

The observations of previous investigators that epinephrin strongly inhibits the activity of the gut was readily confirmed. It was found that the threshold of this inhibition is surprisingly low. In the most favorable instances, immediately after the removal of the intestinal segments from the animal, a brief but clear-cut inhibition of the spontaneous rhythmic beating was demonstrated in dilutions of adrenalin[11] one part to four or five hundred millions. As a diluent defibrinated blood from an epinephrectomized rabbit or Ringer's solution can be used equally well. As the vitality of the tissue decreases, the threshold gradually rises. With segments kept three or four hours in warm Ringer's solution the inhibition was not secured in greater dilution than one in five millions.[12]

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[11] For these tests a fresh bottle of commercial adrenalin was obtained from the Boston agency of Parke, Davis & Co. This brand of epinephrin has been shown by SCHULTZ (Bulletin No. 61, Hygienic Laboratory, United States Public Health and Marine Hospital Service, Washington, 1910) to test quantitatively true to label. The sample used caused by FRÄNKEL'S method rhythmic contractions in rabbits' uteri in dilutions of from 1: 10,000,000 to 1: 30,000,000 as a maximum, and hence, probably, did not surpass its supposed strength.

[12] *Cf.* HOSKINS, Journal of pharmacology and experimental therapeutics, 1911, iii, p. 93.

In addition to this characteristic inhibition there was frequently observed with dilutions just subminimal to the inhibitory value an increase in the activity of the tissue, and often an increased tonus. Fig. 1 shows a record in which this increase of activity occurred at a dilution of 1: 1,000,000,000 followed by a diminution at 1: 500,000,000. Sometimes the increased activity occurs not in the epinephrin solution itself, but immediately after its substitution by Ringer's solution. The threshold for the stimulating effect varies *pari passu* with the vitality of the tissue just as does the inhibitory effect.

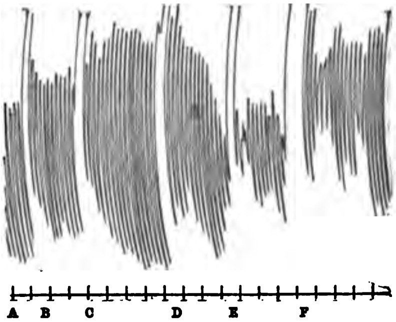

FIGURE 1. — One half the original size. A segment of the small intestine of the rabbit beating in, *A*, Ringer's solution; *B*, Ringer's solution; *C*, Adrenalin 1 : 1,000,000,000; *D*, Ringer's solution; *E*, Adrenalin 1: 500,000,000; *F*, Ringer's, solution diluent in *C* and *E*, Ringer's solution. Time = 30 seconds.

The inhibitory effect of epinephrin upon intestinal peristalsis has been shown by Dixon[13] to be due to a stimulation of the sympathetic myoneural junctions in the tissue. A possible explanation of the augmentation observed is that the metabolic processes of the muscle itself are stimulated by the epinephrin in quantities too small to affect the myoneural junctions, and that the efficiency of the tissue is thereby increased.

The foregoing observations show the futility of attempting to work out the physiology of the adrenal glands by the employment of epinephrin in pathologic quantities. Such attempts involve the assumption that quantitative differences in epinephrin administered to an animal, or secreted by the glands of the animal, will give merely quantitative differences in result, whereas the actual end effect is the algebraic sum of the effects in all the different tissues involved, — tissues which may have different thresholds and different reactions toward epinephrin, — and the total effects of different quantities may vary qualitatively or quantitatively. As a case in point the assumption frequently made that the adrenals have an important part in maintaining normal blood pressure is quite without foundation unless it can be shown that epinephrin within physiologic limits has a pressor effect.

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[13] DIXON: Journal of physiology, 1903, xxx, p. 97.

It seems that the effects of variations in the quantity of circulating epinephrin should be put into two distinct categories, those due to quantities that stimulate the sympathetic myoneural junctions and those below the threshold for such stimulation, — effects that may differ qualitatively as well as quantitatively. If this assumption be true, there is no anomaly in the fact that supranormal doses of epinephrin cause an immediate increase of blood pressure, whereas ligating the adrenals does not cause a corresponding fall. Such observations, in fact, suggest that the peculiar relationship between the adrenals and the sympathetic nervous system may not be a factor in the normal activities of the body, but merely a reserve mechanism for times of special stress.

SUMMARY.

Epinephrin in remarkably high dilutions inhibits peristaltic activities in isolated segments of rabbit's intestine. In still higher dilutions the rhythmic activity of the gut is often augmented. Quantitative differences, therefore, in the amounts of epinephrin acting upon tissues can cause qualitative differences in effect. This fact throws doubt upon conclusions involving the assumption that excessive quantities of epinephrin give merely in increased degree the effects of smaller amounts.

I wish to express my appreciation of the kind permission to use in this research the facilities of the Laboratory of Physiology in the Harvard Medical School.

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ON THE EXCITING CAUSE OF COMPENSATORY MOVEMENTS.

By S. S. MAXWELL.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

IF a shark be rotated around a dorso-ventral axis, compensatory movements of the eyes occur in a direction opposite to that of the rotation. If a frog or a horned toad (*Phrynosoma*) be so rotated, compensatory movements of the head appear. These movements are more or less modified, but are scarcely lessened in intensity if the animal be blinded. They disappear completely if the animal be blinded and in addition suffer section of the auditory nerve. There is no room to doubt the existence of a peripheral end organ or set of end organs in the internal ear capable of giving rise reflexly to the movements in question. Moreover, these movements can be elicited in a very precise way by stimulating, mechanically or electrically, very definite portions of the internal ear, as has been shown by von Cyon, Lee, and others.[1] Thus in the shark excitation of the ampulla of the right horizontal canal causes a turning of the eyes to the left. Excitation of the ampulla of the right anterior vertical canal causes elevation of the right eye with corresponding depression of the left eye and at the same time a rotation of both eyes backward.

The arrangement of the semicircular canals in three planes practically at right angles to each other is very suggestive of an organ for orientation in space or for the mediation of sensations of movement or position. On the basis of this arrangement and on the results of stimulation and of rotation experiments, Lee[2] has developed the well-known theories of Mach and Crum-Brown to a high degree of particularization. The idea underlying these theories is that, on account of the inertia of the endolymph which fills the canals, currents of liquid, or at least changes of pressure, would occur at each movement of the head, and these currents or pressure changes would excite mechanically the nerve endings in the ampullæ. Mach[3] very early abandoned the grosser form of his theory, for he saw that in a tube so small as a semicircular canal no actual current would occur on rotation, although change of pressure could still be supposed to result.

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[1] Maxwell: University of California publications in physiology, 1910, iv, p. 1.

[2] Lee: Journal of physiology, 1893, xv, p. 311; and 1894, xvii, p. 192.

Lyon,[4] at the suggestion of Loeb, however, pointed out an objection to the pressure theory based on the following consideration: If a frog is placed on the turntable with its head toward the periphery and is rotated toward the right, the head is bent to the left. In this case the pressure due to inertia of the endolymph is exerted toward the animal's left. If now the frog be turned so that its head is toward the centre of the wheel and rotation is begun, the pressure of the endolymph is exerted toward the animal's right, but the head is again turned to the left.

I wish to repeat here a still more striking proof that pressure *per se* is not the cause of the excitation which produces the reflex compensation. If an animal be placed near the periphery of a turntable in a tangential position and rotated head foremost in a clock-wise direction, the head will be turned outward, that is, to the left. If, now, the position of the animal be reversed so that its left side is toward the centre and rotated clock-wise (tail foremost), the head is again turned to the animal's left, that is, inward. The pressure of the endolymph may be considered as made up of two components. One of these is tangential, and hence acts equally on the right and left of the animal and so could have no unilateral effect. The other is radial, and is in each case exerted in a direction away from the centre of rotation, that is to say, toward the animal's left when rotated head foremost and to the right when rotated tail foremost. This shows that the radial pressure, the so-called centrifugal force, is not the stimulus which causes the compensatory movements. The above considerations are also in harmony with the fact that a direct movement of translation to right or left evokes not the slightest trace of a compensatory movement.

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[3] MACH: Sitzungsberichte der Wiener Akademie der Wissenschaften, 1874, xviii, part 3, p. 125.

[4] LYON: This journal, 1899, iii, p. 89.

I have been able to show in a still more exact way that the radial force has no perceptible effect as a stimulus to excite the compensatory movements, but that on the other hand the sole effective force is the torsion effect due to the inertia, which Ewald[5] long ago called the *Rotations-Remanens*. The principle of the experiment is as follows: An animal is placed on a turntable and is slowly rotated through an angle of 45 degrees. By a series of trials a rate of rotation — to be more exact, an acceleration—is found which is just equal to the threshold of stimulation. The animal is then placed at some other distance from the axis of rotation and the threshold is again determined. The radial force for any given particle of a rotating body, at a given rate of rotation, varies as the distance of that particle from its centre of rotation. The torsion effect, on the other hand, depends on the angular velocity, but not at all on the radius. If it can be shown that a position on the turntable at a distance from the axis is more effective in evoking the compensatory movements than a position nearer to the centre, then there is evidence that pressure is an effective stimulus. If no such difference exists, the exciting cause must be the torsion effect alone.

The animal used was the lizard, Phrynosoma, commonly known as the horned toad. This is especially convenient for such experiments as Loeb[6] has pointed out, because of the fact that the eyes can be caused to close by merely touching the lids, and retinal reflexes are thus very simply excluded. To facilitate the observation of very weak reactions a light straw as index was attached to the animal's head and projected 80 mm. beyond the snout. A graduated arc was marked on the turntable, and in this way very slight movements could be perceived. Table I shows a specimen experiment. The turntable was rotated by hand through an arc of 45 degrees in each case; the time occupied in that amount of turning was indicated by a stop watch and is set down in column 2. Several trials were made in the one position; then the animal was moved to the other position and a number of trials made. This was done to eliminate possible changes in the excitability due to the effect of handling. The order of the trials is indicated by the trial number in column 1. The response is shown in column 3.

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[5] Ewald: Physiologische Untersuchungen über das Endorgan des Nervus Octavus, Wiesbaden, 1892, p. 148.

[6] Loeb: Archiv für die gesammte Physiologie, 1907, cxvi, p. 368.

TABLE I

EFFECT OF ROTATION THROUGH 45 DEGREES AROUND A VERTICAL AXIS.

Head 25 mm. from centre of turntable.			Head 300 mm. from centre of turntable.		
1 Trial no.	2 Seconds.	3 Compensatory movement.	1 Trial no.	2 Seconds.	3 Compensatory movement.
1	7	Slight.	6	8	Slight.
2	7	Slight.	7	9	None.
3	12	None.	8	9	Slight.
4	10	None.	9	10	None.
5	8	Slight.	10	10	None.
14	8	Slight.	11	8	Slight.
15	8	Slight.	12	7	Slight.
16	8	Slight.	13	6	Strong
17	6	Strong.	22	10	None.
18	5	Strong.	23	9	None.
19	10	None.	24	8	None.
20	9	None.	25	7	Slight.
21	7	Slight.	26	5	Strong.

A second set of observations was made in which the animal was placed upon a board which could be rotated smoothly through 45 degrees about a horizontal axis. A sample experiment is shown in Table II.

It will be seen from inspection of the two sides of Table I that the threshold was reached in this experiment with a speed of about eight seconds for 45 degrees of rotation, and that there is practically no difference produced by changing the distance from the centre. Table II shows similarly that there is no noticeable difference when the animal's head is 25 mm. or 300 mm. from the axis of rotation. Since the radial force (centrifugal force) is expressed by the equation $F = mrw^2$, in which *m* is the mass, *r* the distance from the axis, and *w* the angular velocity, the centrifugal force would be twelve times as great in the one position as in the other. No such difference appears, in fact no difference appears, and hence we are justified in concluding that the excitation is not at all dependent on the radial pressure, but only on the torsion effect, which varies directly with the angular velocity.

TABLE II.

EFFECT OF ROTATION THROUGH 45 DEGREES AROUND A HORIZONTAL AXIS.

Head 25 mm. from axis.			Head 300 mm. from axis.		
1 Trial no.	2 Seconds.	3 Compensatory movement.	1 Trial no.	2 Seconds.	3 Compensatory movement.
1	6	None.	6	6	None.
2	6	None.	7	5	None.
3	5	Slight.	8	4.5	Slight.
4	3	Strong.	9	4	Strong.
5	4.5	Good.	10	5	Slight.
11	5	Slight.	13	5	Slight.
12	4	Strong.	14	6	None.

ANTAGONISM BETWEEN SALTS AND ANÆSTHETICS.— I. ON THE CONDITIONS OF THE ANTI-STIMULATING ACTION OF ANÆSTHETICS WITH OBSERVATIONS ON THEIR PROTECTIVE OR ANTITOXIC ACTION.

By RALPH S. LILLIE.

[*From the Physiological Laboratory, Department of Zoölogy, University of Pennsylvania.*]

SUBSTANCES which act as anæsthetics, *i. e.*, which render irritable tissues temporarily irresponsive to stimuli or less responsive than normally, or which decrease or abolish reversibly the activity of automatically active tissues like the heart, may belong to the most diverse chemical classes. They may be products of normal tissue metabolism; thus carbon dioxide in certain concentrations acts as a typical anæsthetic; bile salts also depress the irritability of nerve and muscle; the so-called "fatigue substances" may be regarded as belonging to this class; the accumulation of certain undefined metabolic products in the blood of higher animals leads periodically to a condition of normal or physiological narcosis known as sleep. Many inorganic salts also possess anæsthetic property; those of magnesium have the most general action of this kind and the most typical because the most reversible. The depressant action of potassium salts is well known. Calcium and strontium salts in certain concentrations decrease reversibly the susceptibility of muscle and nerve to chemical and other stimulation.[1] In general, electrolytes have marked influence on irritability. In some cases acid, in others alkali, show typical anæsthetic action. Bethe found alkali ($C_{OH} = 10^{-4}$ n) to check, and acid ($C_H = 10^{-6}$ to 10^{-5} n) to accelerate the rhythmical contractions of the Medusa Rhizostoma.[2] In the vertebrate heart the reverse is usually observed. Yet traces of acid as well as of alkali heighten the twitching seen in frogs' skeletal muscle in pure isotonic solutions of sodium salts.[3] The effects of a given substance may thus vary from tissue to tissue and from organism to organism, and the same substance in the same concentration may in some cases heighten, in others lower, the irritability of the tissue. Pure solutions of non-electrolytes like sugar show a characteristic anæsthetic action on muscle and nerve.[4] This condition seems general for these tissues, whose irritability normally depends on the presence of sodium salts in their media. It is thus evident that anæsthetic or narcotic action is confined to no class of substances; it is perhaps not too much to say that almost any substance, unless its action is too energetic and hence essentially irreversible (as mercury and silver salts), may in certain concentrations show anæsthetic action, *i. e.*, may temporarily decrease or abolish irritability.

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[1] After immersion for two to three minutes in isotonic calcium, magnesium, or strontium chloride solution, frogs' voluntary muscle fails to contract, or contracts only slightly, when brought into *m*/8 KCl or mixtures of *m*/8 NaCl and *m*/8 KCl. JOSEPH and MELTZER have also recently demonstrated the anæsthetic action of calcium salts on frogs' muscle and nerve (this Journal, 1911, xxix, p. 1). The action of calcium shows in many irritable tissues a characteristic variation with concentration. BETHE found its omission from artificial sea water to arrest the rhythmical contractions of Rhizostoma; addition of a certain proportion

With many substances in certain concentrations the modification of irritability may be in the opposite direction; the tissue becomes temporarily more sensitive to stimuli than normally. Isotonic solutions of sodium citrate greatly increase the irritability of frogs' muscle

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was favorable to the maintenance of rhythm; when larger quantities were added the rhythm was again arrested (Archiv für die gesammte Physiologie, 1909, cxxiv, p. 561). The action of calcium on the vertebrate heart is similar. Many lipoid-solvent anæsthetics also show a stimulating action in lower concentrations and an anæsthetic action in higher. BETHE, however, found magnesium to show a depressant action from the first; so also aluminium (*Ibid.*, 1909, cxxvii, p. 245).

[2] BETHE: Archiv für die gesammte Physiologie, 1909, cxxvii, p. 219.

[3] J. LOEB: Festschrift für Professor Fick, Braunschweig, 1899, p. 101. A similar stimulating action of both acid and alkali in low concentrations is seen in the ganglion of the heart of Limulus: *cf.* CARLSON: this Journal, 1906, xvi, p. 378.

[4] The anæsthetic action of pure isotonic sugar solutions is shown characteristically in Arenicola larvæ; muscular contractions cease within a few minutes in such solutions, while cilia continue; on return to sea water or appropriate salt solutions contractions are resumed. The anæsthetic action of pure sugar solution is similar to that of magnesium chloride solution, though less pronounced. *Cf.* this Journal, 1909, xxiv, p. 463.

and nerve to mechanical and chemical stimuli;[5] pure isotonic solutions of many other neutral sodium salts have a similar though usually less marked effect.[6] Traces of alkali increase the irritability of vertebrate muscle and nerve; increase in the normal ratio of sodium to calcium in the medium has a similar effect on these tissues, while decrease in this ratio lowers irritability.[7] Some substances increase irritability when present in traces, while exhibiting typical anæsthetic action in higher concentrations; this is true of many lipoid-solvent anæsthetics; alcohol is a good instance.[8] The influence of certain anæsthetics on growth shows an interesting analogy. It is well known to florists that the presence of a little ether in the air accelerates plant growth; in higher concentrations ether inhibits growth and cell division, as Claude Bernard first showed for seedlings; on removal of the anæsthetic growth is resumed.[9] The same substance may thus exhibit diametrically opposite actions at different concentrations. In still higher concentrations most lipoid solvents stimulate irritable tissues strongly, and rapidly cause irreversible or cytolytic alterations, *i. e.*, exhibit a powerful toxic action.[10]

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[5] *Cf.* J. Loeb: this Journal, 1901, v, p. 362. In general those sodium salts which lower the concentration of the calcium ions in the tissue (in the surface layer? *cf.* p. 369 of this paper), heighten the contact irritability, both in nerve and muscle.

[6] Pure isotonic solutions of many sodium salts show this action independently of any influence on the calcium compounds of the tissue. The relative activity of the following salts varies with the nature of the anion in the general order: $NaCl < NaBr < NaNO_3 < NaClO_3 < NaI$ and $NaCnS$ — *i. e.*, increases with increase in the promotion of colloidal dispersion. *Cf.* Proceedings of the Society for Experimental Biology and Medicine, New York, 1910, vii, p. 170.

[7] *Cf.* J. Loeb: Oppenheimer's Handbuch der Biochemie, 1909, ii, p. 124. This rule, however, by no means holds true for all irritable tissues; removal of calcium from the medium depresses irritability and spontaneous activity in Medusæ (Bethe: *Loc. cit.*). Deviation from the optimum proportion of calcium in *either* direction may depress irritability.

[8] The action of traces of anæsthetics (ether, chloroform, chloretone, chloral hydrate) in heightening spontaneous rhythmical activity is well shown in the ganglion of the Limulus heart; *cf.* Carlson: this Journal, 1906, xvii, p. 182. The same is true for cilia, as Engelmann observed. Hamburger has recently found that traces of many lipoid-soluble substances — iodoform, chloroform, benzol, turpentine, chloral hydrate — increase the phagocytic activity of leucocytes, while stronger solutions decrease this activity; analogous observations were made with spermatozoa. Hamburger also points out that a stimulating action in low concentrations is widely characteristic of anæsthetics. *Cf.* Hamburger: Archives Néerlandaises des Sciences Exactes et Naturelles, Série III, B, 1911, i, p. 1. Professor Macfarlane informs me that traces of ether increase the irritability of Mimosæ; higher concentrations produce typical anæsthesia.

[9] C. Bernard: Leçons sur les phénomènes de la vie communs aux animaux et végétaux, Paris, 1878, chapter vii, pp. 259 *seq.*

The narcotizing concentration, in the case of any anæsthetic, varies characteristically for different organisms and for different tissues of the same organism. A concentration of ether or chloroform sufficient to abolish completely the normal neuro-muscular or reflex irritability may leave other active tissues almost unaffected; this, of course, is the condition on which the practical use of anæsthetics depends. A good illustration of relative insensitivity to narcotic action in an active tissue is seen in the cilia of many metazoa; these structures, in correspondence with the characteristic automatic and uninterrupted nature of their activity and their irresponsiveness to stimuli, show marked resistance to the action of anæsthetics.[11] Thus the cilia of Arenicola larvæ continue their activity for hours in isotonic sugar or magnesium chloride solutions, or in solutions of chloroform or ether which prevent completely all muscular movement. Analogous differences obtain between different organisms. Some are easy, others difficult to anæsthetize. It is noteworthy that a given anæsthetic produces different effects with different organisms; thus chloretone is a favorable anæsthetic with cats or rabbits, but not with dogs. Individual differences in susceptibility to narcotic action are also well marked; the effects vary with age, — alcohol and anæsthetics affect children more rapidly than adults, — also with the congenital constitution and to a certain degree with the habits and mode of life of the individual. Different men vary in their susceptibility to the narcotizing action of their own metabolic products, *i. e.*, some resist the onset of sleep more readily than others; such persons seem in general to be relatively resistant to other kinds of narcotic action, as of alcohol. This power of resistance can be increased by use, and it has been observed by surgeons that an acquired resistance to alcohol involves also an increased resistance to anæsthetics. A certain modifiability in the physico-chemical constitution — probably more particularly in the lipoid content — of the nerve cells is thus indicated. Wide variation in the quantity and character of the lipoid content of different cells is, in fact, a marked characteristic of highly differentiated organisms;[12] and variation in the susceptibility of different organisms and tissues to narcotic action will no doubt be shown, as research advances, to be the physiological correlative of a corresponding variation in lipoid content.

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[10] I have described this action for Arenicola larvæ in my paper in this Journal, 1909, xxiv, pp. 30, 31.

[11] Compare OVERTON: Studien über die Narkose, Jena, 1901, p. 185: "Zur Narkose von Pflanzenzellen, Protozoën, Flimmerzellen, etc., sind meistens 6–10-fach höhere Concentrationen der indifferenten Narcotika erforderlich als zur Narkose von Kaulquappen."

At the same time certain broad and significant uniformities are found. The chief of these is the almost universal anæsthetic action of substances, which, however diverse chemically, have in common the physical property of dissolving lipoids or of dissolving in lipoids. The connection between fat-dissolving power and anæsthetic action has long been recognized.[13] Overton and Hans Meyer demonstrated in 1899 a parallelism between the narcotic action of various substances and their oil-water partition coefficient. They refer narcotic action to a modification of the lipoids; permeation of the latter cell constituents with the narcotizing substance is the essential condition of narcosis.

That the lipoids are mainly concerned in certain forms of narcosis may be taken as established. The Overton-Meyer theory, however, in so far as it refers narcosis to modification of the lipoids alone, is inadequate because too special. As we have seen, narcotic action is not confined to lipoid solvents; it is merely highly characteristic of them. Why, for instance, do magnesium salts or non-electrolytes exhibit anæsthetic action? The conditions of this similarity of action must be defined in any adequate theory, and in this respect the theory of Overton and Meyer is deficient.

Modification of the lipoids does, however, produce anæsthesia. Why is this? Obviously, in endeavoring to account for a condition so remarkable, we must first consider the probable situation of the lipoids in the cell, since the *locus* of the primary anæsthetic action must correspond to that of the substances whose modification conditions this action. Overton's own work assigns to these substances a special situation; they are to be regarded as concentrated chiefly at the cell periphery, as forming, in fact, an indispensable, perhaps the chief, part of the surface film or plasma membrane. This is shown by the characteristic free permeability of the cell to lipoid-soluble substances; such substances, as a rule, gain rapid entrance; others, chemically similar but lipoid-insoluble, enter slowly or imperceptibly. Lipoid-solvent substances also rapidly modify the osmotic properties of the cell; such substances have a specific action in altering the plasma membrane: even in low concentrations they rapidly destroy the osmotic equilibrium and produce cytolysis. The presence of lipoids in the surface layer must thus be granted; it seems clear also that the state of the lipoids determines the characteristic osmotic properties of this layer. Narcotic action, therefore, if it is identified with lipoid-modifying action, must consist primarily in an alteration of the surface film or plasma membrane of the irritable element. How is this effect possible? Why should modification of the plasma membrane alter the irritability of the cell and under certain conditions abolish all power of response?

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[12] *Cf.* the graphic representation of the relative lipoid and protein content of different tissues in W. Koch's paper, "Recent studies on lipoids," in Journal of the American Medical Association, 1911, lvi, p. 799.

[13] First by Bibra and Harless in 1847. For a historical account of the theories of anæsthesia and narcosis, *cf.* Overton: *Loc. cit.*

To answer this question it is necessary to consider the relation of the plasma membrane to the power of response or irritability of the cell. Recent research has shown that this relation is very intimate. In electrical stimulation a change in the electrical polarization of the semi-permeable membranes has been shown by Nernst and his successors, Lucas, Hill, and others, to be the essential or critical event. Theoretically, changes in the polarization of the membrane may result from either of two general causes: (1) changes in the ionic content of the media in contact with the membrane, — this includes, as Nernst showed, the special case of the action of an external current; or (2) modification in the membrane itself, particularly in its ionic permeability or in its power of combining or liberating ions. Normally we find in an irritable tissue a potential difference between the exterior and the interior of the cell or element (equal in muscle to about 0.1 volt), the outer surface being positive; this potential difference appears dependent on the normal impermeable condition of the plasma membrane, since it diminishes or disappears when this structure undergoes marked increase in permeability, as at death or under the action of cytolytic substances. In other words, the membrane is the seat of a potential difference when impermeable, as normally during rest; this potential difference decreases markedly if the permeability is increased. Now, the potential difference also decreases during stimulation, to return to the normal when stimulation ceases; the effect, in brief, is the same as would be observed if the permeability of the membrane were suddenly and reversibly to increase, and it is difficult to explain it on any other hypothesis. The conclusion we are led to is that the permeability of the plasma membrane undergoes reversible increase during stimulation; stimulating agencies, on this view, are those which increase temporarily, to a sufficient rate and degree, the permeability of the plasma membrane. Direct evidence that an increase of permeability is a normal accompaniment of stimulation is seen in the mode of response of motile plant mechanisms and in various other facts of comparative physiology.[14]

The facts of anæsthesia, interpreted in terms of this theory, indicate that altering the condition of the lipoids in the plasma membrane renders this rapid increase of permeability difficult or impossible under the usual conditions of stimulation. Direct evidence that lipoid solvents prevent an increase in permeability at the same time that they prevent stimulation has hitherto been lacking. Höber has found that in the presence of anæsthetics the action of salts like potassium or rubidium chloride in producing a local negative variation in muscle is checked; and he has interpreted this fact to indicate that stimulation is associated with an alteration of the lipoids, this change being checked or prevented by lipoid solvents.[15] If the local negativity is the expression of local increase in permeability, his observations indicate that anæsthetics render this change difficult, *i. e.*, have an action antagonistic to that of the salt. It can, in fact, readily be shown that anæsthetics may counteract the stimulating action of salts; the twitching shown by voluntary muscle in pure isotonic solutions of sodium salts may be prevented not only by calcium and magnesium salts but also by anæsthetics. I have also found during the past summer that the permeability-increasing action of isotonic sodium salt solutions is checked by anæsthetics simultaneously with the prevention of the stimulating action. This can readily be shown in the pigmented larvæ of Arenicola. As I have already described at length, the stimulating action of pure solutions of sodium and other salts is in this organism associated with the visible permeability-increasing action, seen in the exit of pigment from the cells during the period of strong stimulation. Salts like magnesium or calcium chloride, which check the one effect, also check the other.[16] Marked and rapid increase of permeability appears invariably to involve stimulation. Magnesium salts have an opposite action to sodium or potassium salts; instead of increasing permeability they appear rather to decrease it;[17] at the same time they exert a powerful anæsthetic action. Such facts suggest the view that the essential action of anæsthetics as a class is to *decrease* the normal permeability, thus rendering the increase of permeability requisite for stimulation more difficult. In fact, larvæ transferred from isotonic magnesium chloride solution to $m/2$ NaCl undergo neither stimulation nor loss of pigment.[18] The same is true for larvæ brought from sea water into appropriate mixtures of sodium and magnesium chloride. The facts to be presented below show a precisely analogous action in the case of the lipoid-solvent anæsthetics. Not only the stimulating and permeability-increasing action, but also the toxic action of pure isotonic sodium chloride solution, is markedly diminished by the presence of the lipoid-solvent anæsthetic in the solution in favorable concentration. There is thus clear evidence, at least in the case of this organism, that the anæsthetic interferes with the stimulating action of neutral salts because of its preventing the rapid increase in permeability which in its absence the salt solution produces; also that the prevention of this effect involves a certain protective or antitoxic action.

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[14] I have recently attempted to summarize and co-ordinate the main facts and theoretical considerations which indicate that the primary or critical change in stimulation is a rapid and reversible increase in the ionic permeability of the plasma membrane, in this Journal, 1911, xxviii, p. 197.

[15] Höber: Archiv für die gesammte Physiologie, 1907, cxx, p. 492. *Cf.* also Physikalische Chemie der Zelle und der Gewebe, 3d ed., 1911, pp. 488 *seq.*

EXPERIMENTAL.

During the past summer at Woods Hole I have investigated the action of various lipoid-solvent anæsthetics in conjunction with isotonic solutions of neutral salts of sodium and potassium, using the larvæ of Arenicola and the unfertilized eggs of starfish and sea urchins.

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[16] *Cf.* this Journal, 1909, xxiv, pp. 23 *seq.*; 1911, xxviii, pp. 210 *seq.*

[17] *Loc. cit.*, p. 25.

[18] This Journal, 1909, xxiv, pp. 480 *seq.*

These experiments have had reference not only to the anti-stimulating action of anæsthetics, but also to their antitoxic action. If pure solutions of, *e. g.*, sodium salts stimulate because of their producing abnormal increase of permeability, and act destructively or as toxic agents for the same reason, — loss of the normal impermeability of the plasma membrane involving abnormal interchanges, with loss of essential protoplasmic constituents as well as of the all-important electrical polarization of the membrane, — any substance which prevents the stimulating action of such solutions should at the same time diminish their toxic action. This is true for calcium and magnesium salts, which when present in small proportions prevent the excessive stimulation and loss of pigment produced in Arenicola larvæ by pure solutions of sodium salts, and at the same time exercise a marked antitoxic action. To a certain degree it is also true for anæsthetics, as the experiments described below will illustrate. In the present paper the results of experiments with Arenicola larvæ will be described. Those with unfertilized eggs will be described in a separate paper.

The larvæ of Arenicola are favorable test objects or physiological indicators for the study of the relations between changes of permeability and stimulation because of the presence within the cells of a yellow pigment which diffuses freely into the medium when the permeability is markedly increased. Rapid increase of permeability sufficient to cause exit of pigment[19] is invariably associated with intense stimulation, as shown by strong and persistent muscular contraction. This effect is produced in a typical manner by pure isotonic sodium chloride solutions. In the experiments described below I have studied the modifications which various anæsthetics produce in this action.

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[19] This action is cytolytic, and these experiments may be regarded as indicating merely that strong cytolytic action incidentally involves stimulation. The existence of such a constant connection between these two processes is, however, highly significant. What I have maintained is that increase in permeability is the determining condition of stimulation *as well as of cytolytic action*. Conditions which produce marked and rapid increase of permeability may hence have both effects. In general, cytolytic action results when the increase of permeability decidedly exceeds the normal and is essentially irreversible; normal stimulation when the change is temporary, rapid, variable in degree, and completely reversible. Cytolytic substances, in fact, typically produce contraction in frogs' voluntary muscle, but the effect is characteristically irreversible. Naturally, gradations exist between complete and incomplete reversibility of effect; an otherwise normal increase of permeability, if not soon reversed, may lead to irreversible

In studying the action of volatile anæsthetics like ether the method used was as follows. The larvæ, which are positively phototactic, are allowed to gather on the light side of a watch glass. Large masses are thus easily collected. Approximately equal quantities are then transferred with pipettes to clean, dry 50 c.c. Erlenmeyer flasks corresponding in number to the several solutions used in the series. After the larvæ have gathered in a mass on the light side of a flask, most of the sea water may be poured off and the remainder largely removed by filter paper without disturbing the larvæ. The solutions to be examined are previously prepared and kept in tightly corked flasks; 20 c.c. of each is added to the larvæ-containing flasks and the latter are instantly corked. The immediate effect of any solution may be readily observed with a strong hand lens or dissecting lens through the bottom of a flask. The larvæ average about 0.3 mm. in length, and there is no difficulty in observing the muscular movements under these conditions; evidence of ciliary movement is seen in the swimming movements; if the ciliary movements are too weak to propel the larvæ and cannot be detected with the hand lens, the low power of the compound microscope may be used, or larvæ may be transferred with a pipette to a watch glass and examined rapidly before the anæsthetic has had time to evaporate. The degree of toxic action of a solution is indicated by the degree of revival of muscular or ciliary movement in larvæ transferred after varying intervals to normal sea water.

The effects of solutions of anæsthetics on Arenicola larvæ vary greatly with concentration. Solutions of ethyl ether in sea water in concentrations above 2 vol. per cent (2 c.c. ether plus 98 c.c. sea

changes in the cell; many substances, in fact, produce irreversible or cytolytic effects only when their action is too prolonged. These considerations indicate the general nature of the relationship between stimulating and cytolytic action in the case of any substance; they also indicate why excessive stimulation is injurious to the cell. It is possible that "fatigue substances," like many lipoid solvents, are anæsthetic in lower concentrations and cytolytic in higher.

water) produce strong contractions with loss of pigment; the toxic action is also marked. For characteristic reversible anæsthesia the concentration should not exceed 1 vol. per cent; 0.5 vol. per cent checks but does not entirely abolish muscular movement. Similar conditions hold for other anæsthetics; the favorable anæsthetic concentration, which produces complete muscular immobility without loss of pigment or other immediately injurious action, has to be determined empirically for each substance. It is characteristic that in a concentration of anæsthetic sufficient to abolish muscular contraction completely, the cilia continue their activity, sometimes for hours. The various anæsthetics show characteristic differences of action; thus ether is decidedly more injurious to cilia than chloroform, chloretone, or chloral hydrate.

If Arenicola larvæ are brought directly from sea water into a series of differently concentrated solutions of ether in .55 m NaCl, strong and persistent muscular contractions accompanied by well-marked loss of pigment are seen in the stronger and weaker solutions as well as in the pure .55 m NaCl. But in solutions of a certain intermediate concentration relatively slight and transient contractions occur and there is little or no immediate loss of pigment. If larvæ are left in such solutions for some hours and are then re-transferred to sea water, contractions are found to return much sooner and more completely than after a similar stay in .55 m NaCl. There has been, in other words, a certain protective or antitoxic action.

Table I gives a summary of a series of such experiments.

The characteristic stimulating and permeability-increasing action of isotonic NaCl solution is thus greatly diminished in the presence of 1 vol. per cent ether. The immediate toxic action is also greatly lessened. Contractions return promptly on transfer to sea water after one and a half hours in the solution; even after twenty-four hours most larvæ show partial revival. The other solutions are less favorable, producing at first marked stimulation and increase of permeability and showing less protective action. Distinct protection is, however, seen in all, if the period of exposure is not too prolonged. Thus, after one and a half hours in Solution 2 there is prompt and well-marked recovery in sea water; but after three and a half hours the larvæ fail to revive, although partial recovery occurs after a similar exposure to the pure solution. This indicates that solutions containing

TABLE I.

Solution.	Immediate effect.	Effect of re-transfer to sea water after exposure to solution for the following periods.
1. Pure .55 m NaCl.	Strong and persistent contraction and marked loss of pigment, relaxation gradual, complete in about one minute. Cilia cease and largely break down.	Thirty-two minutes. No contractions for about thirty minutes; later they become well marked. No revival of cilia. About two hours. Motionless for about one hour, feeble contractions later in most. Three and a half hours. After two and a half hours only slight and infrequent contractions. Twenty-four hrs. No recovery.
2. .55 m NaCl + 2 v. % ether.	Strong contraction with marked loss of pigment. Cilia cease and break down.	One hour and twenty-five minutes. Slow contractions in a few seconds, later becoming marked. Three and a half hours. No recovery.
3. .55 m NaCl + 1.5 v. % ether.	Strong contraction, but relatively rapid relaxation. Moderate exit of pigment, decidedly less than in Solutions 1 and 2. Cilia checked at first, but become active later; cease within one hour.	One hour and twenty-three minutes. Contractions return at once, later all show active contractions. Three and a half hours. Well-marked contractions return after a considerable delay. Twenty-three and a half hours. No recovery.
4. .55 m NaCl + 1 v. % ether.	Larvæ contract on contact of solution, but relax at once. Almost no exit of pigment. Cilia cease at first, but revive in two to three minutes; cease again within one hour.	One hour and twenty-five min. Active contractions at once. Three and a half hours. Contractions return after a delay of about twenty minutes, later they become vigorous and frequent. Twenty-three hours. Contractions return gradually in a large portion.
5. .55 m NaCl + 0.7 v. % ether.	Well-marked contraction, but relaxation prompter and loss of pigment less than in the pure solution.	Three hours and twenty-five minutes. Contractions return gradually, next day all show well-marked movem'ts.
6. .55 m NaCl + 0.5 v. % ether.	Shows no distinct difference from the pure solution.	Four hours and twenty min. Recovery is slight, but greater than after similar exposure to the pure solution.

this proportion of ether are at first less destructive to the tissues than the pure solution, though later they become more so. It is noticeable that after twenty-four hours in Solution 2 swelling and general disorganization are more advanced than in the pure solution; while in Solution 4 (with 1 vol. per cent ether) these changes are distinctly lessened. The cytolytic action of the pure solution is thus checked by ether in the lower concentration, while in the higher the cytolytic action of the ether is added to that of the salt and disintegration is accelerated.

In the above experiments the larvæ were transferred directly from normal sea water to the ether-containing salt solution. If they are first anæsthetized in sea water and then brought into the salt solution, the stimulating and permeability-increasing effects are prevented much more completely, even with only 0.7 vol. per cent or 0.5 vol. per cent ether, and the protective action is still more striking.

The following experiments will illustrate:

June 20, 1911, 10.05 A. M. Arenicola larvæ are brought into a solution of .7 vol. per cent ether in sea water. Muscular contractions cease at once, cilia continue. There is no trace of pigment extraction. After seven minutes (10.12) larvæ are brought from the above solution into .55 m NaCl containing .7 vol. per cent ether. There is no visible effect at first, — neither contraction nor loss of pigment. The larvæ remain elongated and rigid and continue slowly swimming by their cilia. In five hours the cilia have largely ceased; the larvæ are then extended and motionless; there is no trace of pigment in the solution.

After five hours in the solution (at 3.14) the larvæ are transferred to sea water. Slight muscular contractions reappear in a few seconds, the cilia also revive. In one hour all show well-marked muscular and ciliary movements. Next day, eighteen hours later, all show active muscular movements; the cilia are also active in most.

Control. — Larvæ transferred immediately from sea water to .55 m NaCl (about 10.00 A. M.) show instant prolonged contraction with loss of pigment. The cilia cease movement and largely disintegrate within a few minutes.

Transfer to sea water after five hours in the pure solution (3.24) produces no immediate effect. Two hours later (5.44) slight muscular contractions are seen at intervals in a few larvæ, but no ciliary movement. Next day almost all are motionless and apparently dead; faint movements are seen in one or two.

It is evident, on comparing the above two series of experiments, that when transfer is made directly from sea water to the ether-containing salt solution, the salt may produce well-marked increase of permeability and stimulation before the anæsthetic action of the ether has had time completely to assert itself; these effects are, however, checked and rapidly reversed, as seen in the prompt relaxation and the relatively slight loss of pigment, and also in the protective action. If the organisms are previously anæsthetized in sea water, the ether-containing salt solution has no evident action at first and only gradually asserts its toxic action. It is noteworthy that under these conditions the cilia remain active for some hours in the ether-containing salt solution, whereas, without previous anæsthetization they cease movement comparatively soon, though more gradually than in the pure salt solution. When it is considered that pure isotonic sodium chloride solutions rapidly disintegrate these structures, the efficacy of the protective action of the anæsthetic will at once be apparent.

Larvæ were also transferred from sea water containing 0.7 vol. per cent ether to pure 0.55 m NaCl. A moderate contraction results at first with slight loss of pigment. After five minutes considerable pigment is extracted on shaking; after twenty minutes the solution contains almost as much pigment as the control (in which larvæ were brought directly from normal sea water into 55 m NaCl). The injury to cilia and muscle is, however, more gradual than in the control; slight muscular contractions persist after one and a half hours in the solution and a few cilia remain active. The immediate toxic action of the pure salt solution is thus somewhat lessened if the larvæ are brought into it while in a state of anæsthesia. Rapid action thus seems an essential condition of the marked injury produced by direct transfer to the salt solution. In the presence of the anæsthetic the action is retarded and the injury is less far-reaching. This experiment is analogous to the one cited above in which larvæ were brought from isotonic magnesium chloride into sodium chloride solution. In this case, however, the stimulating and destructive effects are prevented to a much greater degree than in the experiment with ether just described.

Solutions of other anæsthetics in sodium chloride solution produce effects essentially similar to the above, although their precise nature varies from substance to substance, each showing characteristic specific peculiarities of action. The main results of my last summer's experiments may be briefly summarized as follows:

Chloroform. — In concentrations of one eighth to one tenth saturated in sea water, chloroform anæsthetizes the musculature rapidly and completely without any extraction of pigment. The cilia remain active in such solutions and the larvæ gather in groups or clumps. One sixth saturated solutions cause at first some loss of pigment, but otherwise show similar action. Prompt recovery of contractions follows return to sea water even after many hours in such solutions. In higher concentrations chloroform produces well-marked loss of pigment and contraction, the cytolytic and toxic effects becoming more pronounced as the concentration increases. Saturated solutions are almost instantly fatal.

Larvæ transferred directly from normal sea water to .55 m NaCl one sixth saturated with chloroform contract strongly at first, but almost instantly relax; there is a slight loss of pigment, but much less than in the pure solution; ciliary movement continues, and the larvæ swim actively for some time; no muscular contraction is seen during the stay in the solution. Chloroform has decidedly greater protective action on cilia than ether. Even after eighteen hours in the above solution slow ciliary movement persists in many larvæ. The protective or antitoxic action on the muscle cells is also very decided; transfer to sea water after eighteen hours in the solution is followed within a few minutes by a well-marked revival of muscular contractions; later active contractions are renewed in all larvæ. Similar exposure to the pure .55 m NaCl is completely fatal to the vast majority of larvæ.

Ethyl alcohol. — Larvæ brought from sea water into .55 m NaCl containing 5 vol. per cent alcohol show only slight temporary contraction followed by rapid relaxation; there is no appreciable loss of pigment; even after five hours the solution remains colorless. Muscular contraction ceases instantly, ciliary movement within ten minutes. On return to sea water after one and three fourths hours the cilia revive at once; muscular contractions are resumed after a delay of about ten minutes. After three and a half hours in the solution there is partial revival of cilia on return to the sea water; muscular contractions reappear gradually but completely. After five and a half hours the cilia are destroyed; active muscular contractions return in sea water, though gradually. After twenty-two hours in this solution the pigment is largely extracted, and there is no recovery in sea water. Alcohol thus shows decided antitoxic action at first, in correspondence with its marked effectiveness in checking increase of permeability and stimulation; later it shows itself less effectual than ether or chloroform in protecting against the more prolonged action of the salt solution. After a day in the alcohol-containing solution the larvæ are all dead and partly depigmented.

Chloretone. — Solutions of this substance in .55 m NaCl also prevent the immediate stimulating and permeability-increasing action. Chloretone shows the most striking action of any anæsthetic so far studied. The following experiments will illustrate. Larvæ were transferred directly from sea water to the following solutions. The effects observed were as follows:

1. *Saturated solution of chloretone in .55 m NaCl.* — There is no immediate contraction or loss of pigment, but in half a minute the larvæ shorten to two thirds length, and loss of pigment begins; in three minutes the solution is tinged slightly yellow; within an hour it is deeply colored, and the larvæ show marked disintegration.
2. *.55 m NaCl half saturated with chloretone.* — No contraction and no loss of pigment; the solution remains perfectly colorless for several minutes; the cilia remain slowly active. In ten minutes the solution is faintly yellow; by forty minutes it has the same color as the control; the larvæ are now motionless and begin to show signs of disintegration. Return to sea water after an hour in this solution has no reviving effect.
3. *.55 m NaCl one fourth saturated with chloretone.* — No contraction or loss of pigment; after an hour the solution is still colorless; the larvæ are then rigid, but otherwise normal-looking; the cilia are slowly active. In two and a half hours some pigment is extracted; the cilia have ceased by then. Transfer to sea water after forty-nine minutes is followed by complete revival of muscular contractions. Similar transfer after two and a half hours produces only imperfect and partial recovery; next day the majority of larvæ are found dead and disintegrated.
4. *.55 m NaCl one sixth saturated with chloretone.* — No contraction or loss of pigment. After two hours the solution is still colorless; the larvæ are rigid, with slowly active cilia. Larvæ returned to sea water after two hours show perfect recovery of muscular contractions. After seventeen hours in the solution many revive in sea water, though imperfectly.

The above experiments show that with the four anæsthetics, ether, chloroform, alcohol, and chloretone, the onset of the anæsthetic action is so rapid as to prevent or greatly check the stimulating and permeability-increasing action of isotonic sodium chloride solutions, even when the organisms are suddenly transferred directly from sea water to the anæsthetic-containing salt solution.

Other anæsthetics have a more gradual action and are unable to prevent the initial stimulation and increase of permeability, although they may afterward show distinct protective or antitoxic action. Chloral hydrate, ethyl urethane, benzol, toluol, and xylol belong to this class.

Chloral hydrate. — In isotonic NaCl solutions containing chloral hydrate in the concentrations 0.8, 0.5, 0.3, 0.2, and 0.1 per cent the characteristic persistent strong contraction and loss of pigment occur essentially as in the pure solution. In the 0.3 per cent and 0.2 per cent solutions considerable protective action is seen both for muscle and cilia; recovery in sea water after four or five hours in these solutions is decidedly more complete than after the same exposure to the pure solution.

Ethyl urethane. — Similarly with urethane in the concentrations 0.6, 0.5, 0.4, and 0.2 per cent. The typical contraction, loss of pigment, and destruction of cilia result when larvæ are brought directly from sea water into these solutions. Some protective action was observed in 0.4 per cent and 0.2 per cent urethane solutions, but not in the others.

Benzol, xylol, toluol. — Solutions of these aromatic hydrocarbons in 0.55 m NaCl, in saturated, two thirds saturated, and half saturated concentrations, also produce in all cases marked contraction and loss of pigment. The recovery of muscular contractions in sea water after two and a half hours in the two thirds and half saturated solutions is more complete than after a similar stay in the pure salt solution. Even after five hours in the two thirds saturated solutions distinct protective action is seen. The destruction of cilia by the salt solution is also checked. The saturated solutions show a certain protective action during the first hour, but later prove more toxic than the pure salt solution. The differences between the three hydrocarbons are inessential.

Experiments on the action of anæsthetics in conjunction with solutions of other sodium salts have not yet been tried. Pure isotonic solutions of such salts have an action on Arenicola larvæ similar to that of the chloride, and their stimulating and permeability-increasing action is similarly checked by calcium, though to a degree varying with the nature of the salt.[20] It is thus to be expected that in their relation to anæsthetics they will also be found similar to the chloride.

Anæsthetics and potassium salts. — Isotonic KCl solutions produce in Arenicola larvæ an intense persistent muscular contraction accompanied by marked loss of pigment; the latter effect is distinctly more pronounced than with NaCl. If equal quantities of larvæ are brought from sea water into equal volumes of the two solutions, the .55 m KCl is invariably found, after the first minute, to be decidedly more deeply tinged with pigment than the .55 m NaCl. In correspondence with this difference it is found that the stimulating action of KCl is decidedly the stronger of the two; this is proved by the fact that the above anæsthetics are unable to counteract it to more than a slight degree. The following experiments will illustrate:

July 2, 1911. Larvæ were transferred as above from sea water to the following solutions: (1) 0.55 m KCl, (2) 0.55 m KCl + 1 vol. per cent ether, (3) 0.55 m KCl + ⅙ saturated chloroform, (4) 0.55 m KCl + 5 vol. per cent ethyl alcohol. In all four solutions there occurred at once the characteristic intense muscular contraction with simultaneous loss of pigment; the only evident effect of the anæsthetic was a somewhat less gradual relaxation in Solutions 2 and 3; the immediate loss of pigment was also somewhat decreased in Solution 2.

Though the immediate action of KCl is more intense than that of NaCl and hence shows little or no counteraction by the anæsthetic, a certain protective action is nevertheless shown later; larvæ transferred from Solutions 2 and 3 to sea water after intervals of respectively two and a half, six and a half, and twenty hours showed more complete recovery than after the same exposures to the pure solution. After twenty hours in pure .55 m KCl the larvæ are swollen and partly disintegrated, and there is no revival of contractions in sea water; in solutions containing ether and chloroform as above the swelling and disintegration are distinctly less than in the pure solution, and a certain proportion of larvæ recover contractility in sea water. Alcohol, on the other hand, showed no definite antitoxic action.

As with sodium chloride, the immediate effects of the anæsthetic-containing .55 m KCl are less pronounced if the larvæ are anæsthetized in sea water previously to being brought into the solutions. Larvæ transferred from sea water containing 0.6 vol. per cent ether to pure .55 m KCl contract and lose pigment, but there is a distinct delay in both effects; if similarly transferred to KCl solutions containing ether, chloroform, or alcohol, as above, the delay is much greater; there is a relatively gradual contraction beginning after a few seconds, and pigment diffuses slowly into the solution; the latter is well tinged with yellow after a few minutes, but less so than the pure solution; later this difference disappears, and the protective action is found to be no greater than in larvæ brought directly from normal sea water into the same solutions.

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[20] *Cf.* this Journal, 1911, xxviii, p. 212.

Thus the presence of the anæsthetic in KCl solutions, while retarding the stimulating and permeability-increasing action, is not able entirely to prevent these effects, as in the case of NaCl solutions. Evidently the action of the KCl in increasing permeability is more energetic and hence more difficult to counteract than that of NaCl.[21]

Anæsthetic action of magnesium salts. — I have already described this action in several previous papers cited above. Larvæ transferred from sea water to pure isotonic solutions of magnesium salts exhibit neither contraction nor loss of pigment; all muscular movement ceases in a few seconds, though the cilia continue their activity. If larvæ are then transferred from such solutions to pure .55 m NaCl, little or no contraction results and there is no loss of pigment. Such larvæ are also less rapidly injured by the pure NaCl solution; *i. e.*, the previous treatment with magnesium chloride has well-marked protective effect. Transfer of normal larvæ from sea water to appropriate mixtures of sodium and magnesium chlorides, or sodium and calcium chlorides, produces only slight stimulation unaccompanied by loss of pigment, and in such solutions the typical antitoxic action is seen. These effects are directly analogous to those seen with lipoid solvents.

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[21] Rather singularly, although the immediate stimulating and permeability-increasing action of isotonic KCl solution is more pronounced than that of NaCl (a fact agreeing with the conditions in vertebrate muscle), prolonged exposure (some hours) to $m/2$ KCl is less injurious than to $m/2$ NaCl (*cf.* my paper in this Journal, 1909, xxiv, p. 459, Tables IX and X, pp. 488, 490). Possibly the immediate strong action of the KCl is succeeded by a secondary permeability-decreasing action which retards further breakdown for a time. In lower concentrations potassium depresses irritability without toxic action.

The rapid increase of permeability with the associated stimulating and toxic action is checked or prevented by the magnesium or calcium or other antitoxic salt.

CONCLUSION.

The foregoing experiments indicate clearly the fundamental importance of the limiting layer or plasma membrane in anæsthesia. So far as my observations with Arenicola larvæ have extended, the intensity of the stimulating action produced by salt solutions shows a definite parallelism with the degree of permeability-increasing action as indicated by the liberation of pigment from the cells. Substances, whether salts or "anæsthetics," which check or prevent the permeability-increasing action of the pure salt solution also check or prevent stimulation by this solution.[22] These experiments also indicate clearly that the toxic action exerted by the pure salt solution is due in large part to the destruction of the normal semi-permeable properties of the plasma membrane, since prevention of the initial rapid increase of permeability has, together with the anti-stimulating, a marked protective or antitoxic action. There is thus experimental evidence that anæsthetics produce their characteristic effects not by entrance into the cell, but by a characteristic surface action, consisting in an alteration of the normal properties of the limiting surface film or plasma membrane of the cell.[23]

The nature of the general theory of anæsthesia to which the foregoing facts and considerations directly point will be already evident. Anæsthetics are substances which render the plasma membrane of the irritable tissue more resistant than normally to changes of permeability. They may at the same time render the membrane even less permeable than in the normal resting state; magnesium chloride appears thus to affect the cells of Arenicola larvæ; but the essential effect is to deprive the irritable elements of their characteristic property of responding to changes of condition by rapid and reversible changes of permeability. This property seems to be the essential peculiarity of irritable tissues. Any substance or condition, such as anelectrotonus, which so alters the membrane as to deprive it temporarily of this property has anæsthetic action.

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[22] It should, of course, be noted that such substances also prevent *normal* stimulation, as shown by their characteristic anæsthetic action in sea water.

[23] Lipoid solvents enter the cell immediately, as OVERTON has shown, and a portion of the anæsthetic action might conceivably be attributed to this entrance. But magnesium salts, which have equally characteristic anæsthetic action, enter the cell very slowly, if at all. Yet anæsthesia is complete after a few seconds in $m/2$ $MgCl_2$ solution. The same is true of isotonic sugar solution, though in this case the action is slower. The essential change in anæsthesia must be *superficial*, in these cases at least.

A complete theory of anæsthesia implies a complete theory of stimulation, and this has not yet been attained. It is agreed, however, that the most constant feature of the latter process is its association with a temporary decrease in the potential difference between the outer surface and the exposed interior of the irritable element. The same effect follows any marked increase in surface permeability, however induced; rapid increase of permeability also produces strong contractions in muscle, as already seen. On the membrane theory the electrical variation of stimulation is simply the expression of a depolarization of the limiting membrane of the irritable element, consequent on temporary increase in ionic permeability. Any sufficient increase in permeability due to cytolytic or other action results in a similar depolarization and hence under certain conditions in stimulation. Rapid depolarization of the plasma membrane thus appears to be the critical or primary event in stimulation. Nernst has shown that electrical stimulation involves a sudden change in the electrical polarization of the semi-permeable boundary surfaces of the irritable tissue; the existence of a demarcation current in the resting tissue during life indicates that these surfaces are normally the seat of an electrical polarization; and the law of polar stimulation indicates that the external electrical current stimulates where its effect is suddenly to diminish this pre-existent polarization. The conclusion follows — if we grant that stimulation involves an increase of surface permeability — that the degree of electrical polarization of the membrane *determines* its permeability, depolarization corresponding to or inducing increased permeability. A similar interdependence between the electrical condition of the membrane and its permeability has been observed by Girard for membranes of pigs' bladder and other materials. The facts of electrical stimulation thus lead directly to the assumption of a reciprocal interdependence between the degree of permeability of the plasma membrane and the degree of its electrical polarization, — variation in the one conditioning a corresponding variation in the other. This seems to be the essential condition on which irritability, or the possibility of electrical or other stimulation, depends.

We are thus led to conceive of the plasma membrane in irritable tissues as a structure having the following essential properties: a thin colloidal surface film, partly lipoid, partly protein, containing electrolytes and other substances (partly in a state of adsorption), highly impermeable (during rest) to dissolved substances of low molecular weight (unless lipoid-soluble); and the seat of an electrical polarization of such a kind that the outer surface has during rest a potential about 0.1 volt higher than the inner. This potential difference is itself an essential factor in determining the permeability of the membrane, — its decrease, even to a slight degree, resulting in an increase in permeability which in highly irritable tissue may be very pronounced and rapid.[24] Such a condition of increased permeability and depolarization will necessarily spread — because of the electrical effects in its immediate neighborhood — from the point of stimulation over the whole membrane.[25]

In anæsthesia it is to be assumed that the membrane is so altered that it fails to respond to a change in its electrical polarization by an increase in its permeability. This failure, as we have seen, may be due to a general action on the colloids of the membrane or to an action confined at first to the lipoids. Why alteration of the colloids should thus alter the properties of the membrane cannot be said at present with any definiteness; it is known, however, that the permeability of colloidal membranes depends on the condition of the colloids, and that this permeability can be altered by electrolytes.[26] Ready reversibility of colloidal aggregation state is presumably a necessary condition of irritability, and the depressant or anæsthetic action of salts of alkali earth or heavy metals may be due to their decreasing the readiness or the reversibility of aggregation changes.[27] It is also far from clear why changing the state of the lipoids should so alter the properties of the membrane. Investigators are not agreed as to the nature of the relations between the lipoids and the other colloids of the cell, — *i. e.*, whether there is combination, simple intermixture, or some definite structural arrangement, mosaic-like or otherwise. The suggestion that the lipoids have a "protective" influence on the other colloids of the membrane — limiting their changes of aggregation state — renders certain facts somewhat more intelligible; *e. g.*, why in the presence of chloroform and ether the isotonic NaCl solution has relatively little immediate action on the membrane and fails to break down the cilia. NaCl and other electrolytes unquestionably increase the permeability of the membrane by altering the state of colloidal dispersion;[28] and this effect is in fact checked or prevented if the tissue is previously impregnated with the anæsthetic.

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[24] The conception of a membrane whose permeability may change reversibly within a period of .001 second or less — as the facts of nerve stimulation indicate — may seem inadmissible to many; yet the difficulty is of the same nature as — and certainly no greater than — the difficulty of accounting for the rapidity of alternate contraction and relaxation in the colloidal fibrils of muscle cells and cilia. If the state of tension of a colloidal fibril can vary so rapidly as we observe to be the case, there is no reason to doubt that the state of ionic permeability of a colloidal membrane can vary with at least equal rapidity.

[25] For a fuller discussion of this point *cf.* my paper in this Journal, 1911, xxviii, pp. 217 *seq.*

[26] *Cf.* ZANGGER's review in Ergebnisse der Physiologie, 1908, vii, pp. 143 *seq.*

With reference to the precise manner in which the state of the lipoids is modified during anæsthesia by lipoid solvents, there are several possibilities to be considered. Any purely solvent action is precluded, since the effect is characteristically reversible; this consideration has been dwelt on by Overton in his book on narcosis.[29] A second possibility is that the state of colloidal dispersion of the lipoid is altered — presumably increased; this, however, seems to be inconsistent with the observation of Koch that there is no relation between the anæsthetic properties of a lipoid solvent and its ability to hinder the precipitation of lecithin by calcium.[30] The third possibility, and the one which seems best fitted to account for the phenomena observed, is that there is no change in the state of dispersion of the lipoid, — *i. e.*, in the *number* of colloidal particles, — but simply an alteration in the relative dimensions of the lipoid and other colloidal particles in the membrane, — *i. e.*, in the volume occupied by these several constituents. The anæsthetic tends to become distributed between the lipoid particles and the other solvents present in accordance with its characteristic partition coefficients between these solvents and water; the accumulation of the lipoid solvent in the colloidal particles of lipoid is thus favored; hence the latter undergo increase in size or swell. The colloidal particles in a lecithin suspension do, in fact, undergo an evident increase in size in the presence of ether or chloroform.[31] This change is reversible, like all changes involving simple solution, and may occur independently of any change in the state of dispersion, — *i. e.*, in the number of particles. Its effect on the membrane will be to alter the structural consistency of the latter in a definite and perfectly reversible manner. It is to be inferred that after the lipoid particles have increased in volume beyond a certain degree the membrane becomes incapable of responding to the stimulating agency by a rapid increase in its permeability as normally. It remains instead permanently impermeable, a condition corresponding to inexcitability.

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[27] The reversibility of the aggregation changes produced by salts in solutions of proteins shows a general decrease in the order alkali, alkali earth, and heavy metal salts. *Cf.* PAULI: Beiträge zur chemischen Physiologie und Pathologie, 1905, vi, p. 233.

[28] The action of the pure isotonic NaCl solution on the membranes is probably essentially similar to its action on the cilia which are visibly broken down or transformed into droplets or granules after a few minutes in the solution.

[29] Page 51.

[30] *Cf.* W. KOCH: Journal of pharmacology and experimental therapeutics, 1910, ii, p. 249.

Certain current hypotheses attribute anæsthetic action to a direct suppression of processes which are rather to be regarded as secondary consequences of stimulation than as inherent in the nature of the stimulation process itself. Thus the view that anæsthetics produce their effects by retarding or preventing intracellular oxidations has been adopted by several investigators, notably by Verworn.[32] The main difficulty with this view is that anæsthesia is as characteristic of nerve, where oxidations are slow and not evidently increased during stimulation, as of muscle, where they are enormously accelerated. Moreover, cyanides, which characteristically suppress oxidations, only gradually lower the irritability of nerve.[33] Again Warburg has shown that sea urchin eggs may be anæsthetized — so as to arrest cleavage

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[31] *Cf.* Calugareanu: Biochemische Zeitschrift, 1910, xxix, p. 96.

[32] *Cf.* Verworn: "Ueber Narkose," Deutsche medizinische Wochenschrift, 1909, No. 37.

[33] *Cf.* Dontas: Archiv für experimentelle Pathologie und Pharmacologie, 1908, lix, p. 430.

— without notably altering the rate of oxidations.[34] On the present view the increase in oxidations in such a tissue as muscle is to be regarded as one of the secondary effects of stimulation; prevention of stimulation necessarily involves a suppression of oxidations in this tissue, but only because an increase in oxidations is here an essential consequence of stimulation. Naturally, all of the effects that normally follow stimulation, including the increase of oxidations, are checked or prevented during anæsthesia. The facts already adduced indicate clearly that the primary anæsthetic action consists in an alteration of the limiting membrane of the irritable tissue. From the general facts of electrical stimulation we must conclude that in muscle rapid changes in the electrical polarization of the membrane involve in some manner a marked acceleration of oxidations; the precise conditions of this effect are insufficiently understood. It is, however, to be regarded as highly significant that during the height of stimulation in voluntary muscle there is a rapid rhythmical fluctuation of the action current,[35] indicating on the present theory a corresponding rhythmical variation in the electrical polarization of the plasma membrane; this would involve similar changes in the electrical polarization of the other electrically polarized surfaces within the system enclosed by such a membrane, and it appears highly probable that the high rate of oxidation in active muscle is to be referred to this condition.[36] Anæsthetics, by rendering impossible such rapid changes of polarization in the membrane, would prevent the increase in oxidations normally following stimulation. On such an hypothesis the manner in which alteration of the plasma membrane may modify the oxidations within the cell, and prevent their increase by stimulating agencies, becomes at least partially intelligible.

[34] WARBURG: Zeitschrift für physiologische Chemie, 1911, lxx, p. 413.

[35] *Cf.* the papers of GARTEN, PIPER, DITTLER, and F. BUCHANAN; for references *cf.* FRÖHLICH: Sammelreferat: über die rhythmische Natur der Lebensvorgänge, Zeitschrift für allgemeine Physiologie, 1911, xiii.

[36] I find that the oxidative formation of indophenol in frogs' blood corpuscles — which, it should be noted, shows a characteristic relation to the nuclear *surface* (*cf.* my paper in this Journal, 1901, vii, p. 420) — is markedly accelerated, especially in leucocytes, by passing induction shocks through a thin layer of suspended corpuscles on a slide. Rapid alternation of polarization and depolarization at the active surfaces evidently furthers this particular oxidation at least. These experiments will be described in a later paper.

SUMMARY.

The chief experimental results and conclusions of the present paper may be briefly summarized as follows:

1. In the presence of ether, chloroform, alcohol, and chloretone, in concentrations corresponding to those which produce typical anæsthesia, pure isotonic solutions of sodium chloride no longer produce in Arenicola larvæ the typical strong muscular contractions, accompanied by marked loss of pigment, which characterize the action of the pure solution. The anæsthetic thus prevents rapid increase of permeability at the same time that it prevents stimulation.

2. The presence of anæsthetics also markedly retards the injurious action of pure solutions of sodium chloride on the muscles and cilia of these organisms. Anæsthetics thus show, in concentrations that produce anæsthesia, a well-marked protective or antitoxic influence similar to that of calcium or magnesium chloride. This influence is also to be referred to a prevention of the cytolytic or permeability-increasing action of the pure salt solution on the plasma membranes.

3. Other anæsthetics — chloral hydrate, urethane, benzol, xylol, toluol — are more gradual in their action; they show a more or less distinct protective or antitoxic influence, but do not prevent the immediate stimulating and permeability-increasing action of isotonic NaCl solutions. On the other hand, isotonic magnesium chloride solutions rapidly anæsthetize the larvæ, and completely prevent stimulation and increase of permeability on subsequent transfer to .55 m NaCl; the protective or antitoxic action is also pronounced.

4. The general conclusion is drawn that anæsthetic action consists essentially in an alteration of the surface film or plasma membrane of the irritable element, of such a kind that the rapid and reversible increase in ionic permeability, which is the primary or critical change in stimulation, is rendered difficult or impossible. The cell is at the same time rendered more resistant to those forms of toxic action which consist in an abnormal increase of surface permeability.

THE SPATIAL VALUES OF THE VISUAL FIELD IMMEDIATELY SURROUNDING THE BLIND SPOT AND THE QUESTION OF THE ASSOCIATIVE FILLING IN OF THE BLIND SPOT.[1]

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HISTORICAL.

THE final draft of this paper will contain a complete *résumé* of the literature of the blind spot. In the present report nothing more elaborate will be attempted than a brief classification of the problems it has presented, with a passing reference to bibliography.

The problems fall under three general headings: (1) the determination of the limits of the blind spot; (2) the spatial values of the region surrounding the blind spot; (3) the filling in of the blind spot. Although our report is directly concerned only with the last two of these problems, brief mention will be made of the first for the sake of historical continuity.

1. *The determination of the limits of the blind spot.* — Three views have been held as to the limits of the blind spot. (*a*) The entrance of the optic nerve over its whole extent is insensible to light. (*b*) The entrance of the retinal artery and vein alone is blind. (*c*) Not only is the entrance of the optic nerve blind, but the area immediately surrounding it is relatively blind.

The first view was proposed by Mariotte,[2] the discoverer of the blind spot in 1668.[3] It was immediately opposed by Pequet,[4] who believed that the defective area is limited to the entrance of the retinal blood vessels. Pequet's view has been supported chiefly by Volkmann and Coccius; and Mariotte's by Hannover, Weber, Listing, Donders, Fick and DuBois-Reymond, and Helmholtz.[5] The third view was

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[1] Read before the Princeton meeting of Experimental Psychologists, April, 1909.

[2] MARIOTTE: Philosophical transactions, 1668, iii, p. 668; 1670, v, pp. 1023–1042.

[3] He thought the retina was continuous across the place of entrance of the nerve, and argued, therefore, that not the retina but the choroid coat is the seat

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of the visual sensation. This inaugurated a discussion that lasted for nearly two hundred years. It did not end until Heinrich Müller (Verhandlungen der physicalisch-medicinischen Gesellschaft zu Würzburg, 1852, iii, pp. 336–340; 1854, iv, pp. 96–100; Sitzungsberichte der physicalisch-medicinischen Gesellschaft zu Würzburg, 1853, p. 234) and Kölliker (Verhandlungen der physicalisch-medicinischen Gesellschaft zu Würzburg, 1852, iii, pp. 316–336) demonstrated the anatomical connection of the nerve fibres with the rods and cones, and developed arguments for considering the rods and cones the light-perceiving elements.

[4] Pequet: Philosophical transactions, 1668, iii, pp. 669–671.

[5] Volkmann (Wagner's Handwörterbuch, 1846, iii, p. 271) calculated from the size of the gap in the visual field and from the projection distance the size of the defective area, which he found corresponded to measurements of the diameter of the retinal artery. His results are opposed to those obtained by Weber (Berichte über die Verhandlungen der königlich-sächsischen Gesellschaft der Wissenschaften zu Leipzig, mathematisch-physische Classe, 1852, pp. 149–152). Weber measured the optic nerve at its place of entrance in two eyes, and got 2.09 mm. diameter in one eye, 1.71 mm. in the other. The blood vessels of these eyes measured .704 mm. and .63 mm. Measurements of the diameter of the blind spot have been made also by Hannover (Das Auge, 1852, p. 72), Listing (Wagner's Handwörterbuch, 1853, iv, p. 492), Fick and DuBois-Reymond (Müller's Archiv für Anatomie, Physiologie und wissenschaftliche Medicin, 1853, p. 405), and Helmholtz (Physiologische Optik, 1896, p. 253). Hannover's estimate of the diameter of the blind spot based upon measurements taken for twenty-two eyes is 1.116 mm. Helmholtz places it at 1.81 mm.; Fick and DuBois-Reymond at 1.3 mm to 1.6 mm.; Listing at 1.55 mm. They all affirm that these measurements correspond much more closely with the diameter of the entrance of the optic nerve than with the diameter of the retinal artery at its place of entry.

Two further arguments have been advanced in support of this view. The first, by Donders (Onderzoekingen gedaan in het Physiologisch Laboratorium der Utrechtsche Hoogeschool, 1853, pp. 133–137) and Helmholtz (*op. cit.*, p. 250) demonstrated directly that the nerve fibres at their entrance into the retina are insensible to light. With the aid of an ophthalmoscope they projected the image of a flame into the eye so that it fell on the nerve fibres as well as on the blood vessels. No sensation was experienced. The second argument, advanced by Weber (*loc. cit.*) and Hannover (*loc. cit.*), is that the blind spot is oval in shape. Were the defective area limited to the blood vessels, it would not have the form of a continuous oval, but would be irregular and branchlike.

Wittich (Archiv für Ophthalmologie, 1863, ix_3, p. 1) and Aubert (Physiologie der Netzhaut, 1865, p. 256) both agree that the optic nerve over its whole extent is insensible to light.

advanced by Griffin.[6] He found that the diameter of the blind spot varies within limits inversely as the intensity of the stimulus. He was supported by Weber,[7] who contended that the margin of the blind spot is partially sensitive to light of great intensity; also by Bjerrum,[8] Meisling,[9] and Groenouw,[10] who found that the area of absolute blindness is surrounded by a zone of relative blindness in which large stimuli are visible but smaller ones are not. On the other hand, however, Johansson,[11] Ovio,[12] Polimanti,[13] and Haycroft,[14] all ascribe to this zone only a degree of blindness to color.

2. *The spatial values of the region surrounding the blind spot.*—That there is no shrinkage of the visual field due to the influence of the blind spot has been held by Volkmann,[15] Fick and DuBois-Reymond,[16] Helmholtz,[17] Wundt,[18] and others who worked with comparatively large stimuli. When superimposed upon the blind spot, they appeared as large, for example, as similar stimuli presented to the corresponding area of the other eye.

3. *The filling in of the blind spot.*—If it is held that there is no shrinkage of the visual field due to the influence of the blind spot, an explanation is required of the fact that no gap is perceived in the monocular field of vision. Up to the present time the theory has been generally held which was advanced independently by Volkmann and by Fick and DuBois-Reymond, namely, that the gap is filled in associa-

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[6] GRIFFIN: London medical gazette, new series, 1838, ii, pp. 223–232.

[7] WEBER: Berichte über die Verhandlungen der königlich-sächsischen Gesellschaft der Wissenschaften zu Leipzig, mathematisch-physische Classe, 1852, p. 149.

[8] BJERRUM: Nordisk Ophthalmologisk, Tidsskrift, 1890, ii, p. 141.

[9] MEISLING: Annales d'oculistique, 1900, cxxiv, p. 430.

[10] GROENOUW: Archiv für Augenheilkunde, 1893, xxvi, p. 125.

[11] JOHANSSON: Upsala Läkareförenings Förhandlingar, 1884, xix, pp. 491–493.

[12] OVIO: Annali di Ottalmologia, a, 1906, xxxvi.

[13] POLIMANTI: Journal de psychologie, 1908, v, p. 298.

[14] HAYCROFT: Journal of physiology, 1910, xl, pp. 492–497.

[15] VOLKMANN: Berichte über die Verhandlungen der königlich-sächsischen Gesellschaft der Wissenschaften zu Leipzig, mathematisch-physische Classe, 1853, pp. 27–50.

[16] FICK and DUBOIS-REYMOND: Müller's Archiv für Anatomie, Physiologie, und wissentschaftliche Medicin, 1853, pp. 396–407.

[17] HELMHOLTZ: Physiologische Optik, 1896, pp. 717–727.

[18] WUNDT: Physiologische Psychologie, 5th ed., ii, 1902, p. 510.

tively by the background.[19] Whether or not a gap in sensation can thus be filled in associatively from the surrounding sense field or by spatial or qualitative ideas, has become a question of considerable theoretical importance, for the principle has been extended to explain certain phenomena in other sense departments. For example, it has been utilized by Helmholtz[20] to explain visual contrast; by other systematists[21] to explain why cutaneous organs, spatially separated, the simultaneous stimulation of neighboring pairs of which are sensed as two, give rise to a continuum of sensation. The wider theoretical bearings of this question and their historical dependence upon the blind spot have constituted our chief incentive to undertake this problem.

EXPERIMENTAL.

The work was done in a well-lighted optics room. The observer was seated before a screen of white drawing paper which was placed at a distance of 67 cm. The head was held firm by means of a mouthboard fastened to an upright standard rigidly clamped to a table.

Early in the work the usual method of closing the unused eye and fixating a point with the eye whose blind spot was to be mapped, was found, to be deficient in two respects. In the first place, the darkened field of vision of the closed eye tended to wash over and obscure the vision of the eye that was being mapped. This operated to give variable limits to the blind area, and otherwise to render the work inaccurate. And, secondly, monocular fixation was found to be very fatiguing and unsteady. Working under these conditions, our observers reported that steady fixation and clear vision could not be maintained long enough to enable them to make accurate judgments. A better method was devised as follows. A piece of white cardboard 8 cm. × 12 cm. was held vertically between the two eyes, thereby cutting off nasalwards the field of vision for each eye. The point was then fixated with both eyes, and the field of vision of the right eye tested for the location of the blind spot by the following method. A stimulus of Hering black paper, 1 cm. square, was fastened on the tip of a cardboard strip and run horizontally temporalwards from the fixation point until the observer reported its disappearance. Starting within the blind area, the direction of movement was then reversed, and the point noted at which the stimulus reappeared. This procedure was repeated several times, and the average of the results obtained was taken as the final determination of the edges of the blind area on the nasal side of the meridian. Other meridians were explored in the same way until the outline of the blind area was completely determined.

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[19] VOLKMANN (*op. cit.*, p. 36), 1853, is usually accredited with the first formulation of the doctrine of the associative filling in of the blind spot. It should be noted, however, that in the same year FICK and DUBOIS-REYMOND (*op. cit.*, p. 398) published independently of VOLKMANN a discussion of the blind spot in which they advance a theory which is essentially the same as VOLKMANN'S. They believe that the gap is filled in by the mind, which in so doing acts in accord with certain laws, the most usual of which is that the sensation on the neighboring retinal elements is carried over the gap.

[20] HELMHOLTZ: *Op. cit.*, p. 564.

[21] See TITCHENER: Experimental psychology, 1901, i, Part II, p. 90.

It has frequently been mentioned in the literature that the limits of the blind spot are not constant even when a stimulus of constant intensity is used. This variability is often attributed to changing conditions of retinal fatigue. The limits of the blind spot were found to vary in our work, but both the experimental evidence and the introspection of the observers indicate that this variability was a function of practice in making the judgment and of steadiness of fixation. If it were due to retinal fatigue, we should expect the size of the blind area to increase on prolonged fixation. This did not take place. Further, if retinal fatigue were a factor of any importance, one might expect the progressive fatigue resulting from a long series of observations to cause the limits of the blind area to become extended. On the contrary, however, it was found that all of the observers tended, within limits, to narrow the blind area as the experiment progressed. This condition is to be expected as a result of practice in fixating and making the indirect vision judgment. In this connection one scarcely need mention that it is a peculiarly difficult feat to hold steady fixation while striving to attend to an object in indirect vision, so closely is visual fixation associated with attention. The practice error is, then, especially large, and we are inclined to assign to this cause rather than to retinal fatigue the varying determinations of the limits of the blind spot when a stimulus of constant intensity is used. The fact that the stability of our determinations was very much increased by the method of binocular fixation which we finally adopted, may also be added as evidence that the variability of limits is a matter of accuracy of fixation and skill in making the indirect vision judgment.

A number of observers, both experienced and inexperienced, have been used in this work. Tables have been compiled from the results of three selected as representative: Misses Chamberlain (C) and Rand (R), Fellows in Psychology, and Rodi (Ri), Graduate Scholar in Psychology.[22] The more essential points, namely, shrinkage, the falling off of the influence of the blind spot as we recede from its edges, and the magnification of spatial values in the zone immediately surrounding the blind spot, have also been verified each year for the past four years in the drill course of the undergraduate laboratory.

The following figures are drawings showing the position, outlines, and dimensions of the blind spot of the right eye of Observers C and R, of the left eye of Observers C and Ri.[23]

RESULTS.

1. *Complete shrinkage takes place when the stimuli observed are on the edges of the blind spot.* — Owing to the variability of the limits of the blind spot, due to unsteady fixation and minor influences, the limits determined at any one time cannot be taken as more than provisional for future determination. If, for example, one wishes to ascertain whether stimuli placed on opposite edges of the blind spot are drawn together or remain separated, he must make anew his determination for each judgment. Moreover, the determination must be made during the same act of fixation in which the judgment is passed. This was accomplished by us as follows. The stimuli were placed on the opposite edges of the blind spot, as located by the previous provisional determination, and the observer was asked to judge what he saw. If he reported two separate stimuli, the stimuli were moved nearer to each other and the observation repeated until the stimuli were judged as one. Then, to make sure that both and not only one were really within the field of vision, each was covered in quick succession to see whether

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[22] As the space available for tables in this Journal is necessarily limited, the detailed data for R will be omitted.

[23] These figures are one-third the actual size of the observer's blind spot at a projection distance of 67 cm.

the other could be perceived. If, as frequently happened, one stimulus had been moved within the blind spot, nothing was seen when the other was covered. But, if each were seen when the other was covered, and only one strip was seen when both were exposed, we were guaranteed that the strip seen was formed by the actual drawing together of the two strips exposed on the opposite edges of the blind area. The stimuli used were made of Hering black paper, 1 cm. × .3 cm. When placed on opposite edges of the blind spot, with their shorter dimension forming a part of the distance judged, they were always seen as one strip varying at different times from .3 cm. to 1 cm. in breadth. This was found to be true whatever meridian was worked with. Hence, we conclude that complete shrinkage takes place when the stimuli observed are situated on opposite edges of the blind spot; or, to reduce the statement to retinal terms, the opposite edges of the blind spot function spatially as contiguous areas.

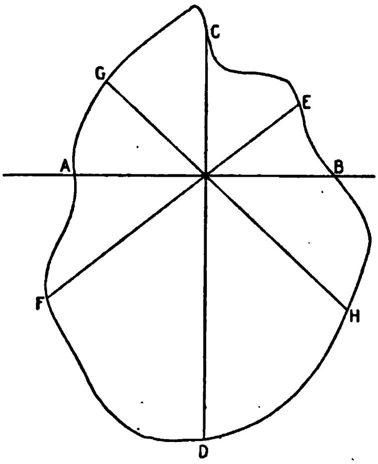


FIGURE 1. — Map of blind spot of observer C (right eye). Projection distance, 67 cm. Fixation point to left of figure. Distance of *A* from fixation point, 29 cm. Distance *A B*, 10.5 cm. Distance *C D*, 15.5 cm. Distance *E F*, 12.5 cm. Distance *G H*, 13.3 cm.

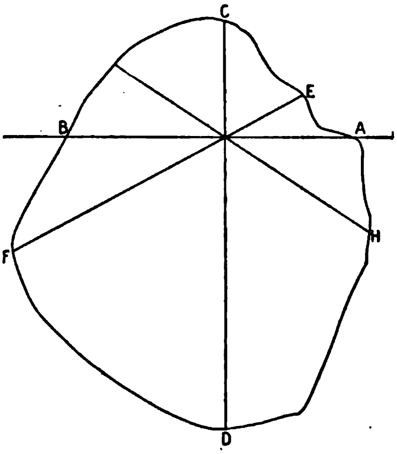


FIGURE 2. — Map of blind spot of observer C (left eye). Projection distance, 67 cm. Fixation point to right of eye. Distance of *A* from fixation point, 27 cm. Distance *A B*, 11.5 cm. Distance *C D*, 16.5 cm. Distance *E F*, 13.2 cm. Distance *G H*, 12.3 cm.

This statement holds, however, only of the edges of the blind spot. As we recede from the edges, we find that the shrinkage falls off rapidly, as the next chapter of our report shows.

2. ***There is a rapid falling off of the influence of the blind spot upon spatial values as the stimuli recede from its edges.*** — Obviously, the solution of our problem is not completed with the demonstration of shrinkage of spatial values for stimuli placed upon the edges of the blind spot. It is required in addition to ascertain the effect of the blind area upon more remote portions of the visual field. Also, the need to explain why previous methods of working had failed to reveal shrinkage gave an additional incentive to continue the investigation. Deficiencies of technique, such as monocular fixation, lack of adequate standards of comparison, and other sources of inaccuracy, varying for the different investigations, are scarcely sufficient to account for a practically uniform verdict of no shrinkage. A survey of the methods, however, showed one feature common to every procedure in which no shrinkage was found, namely, the stimuli observed were in every case considerably larger than the blind area for the projection distance used. This suggested that possibly the influence of the blind spot upon the spatial values of the retina does not extend very far from its edge, in which case it would happen that if the stimulus were sufficiently

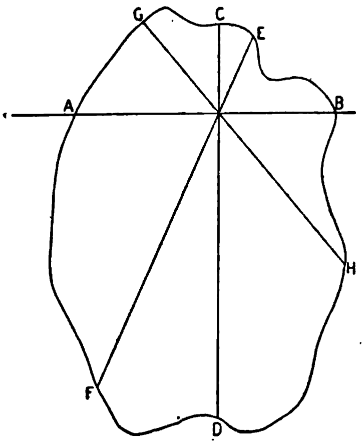


Figure 3. — Map of blind spot of observer R (right eye). Projection distance, 67 cm. Fixation point to left of figure. Distance of *A* from fixation point, 31 cm. Distance *A B*, 10.3 cm. Distance *C D*, 15.6 cm. Distance *E F*, 15.3 cm. Distance *G H* 12.7 cm.

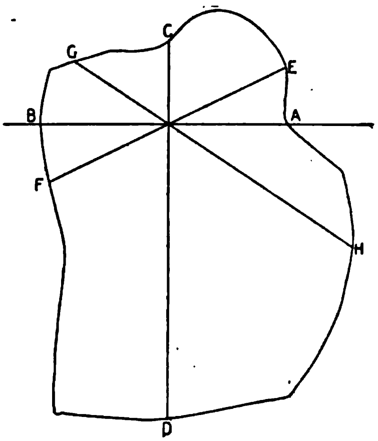


Figure 4. — Map of blind spot of observer Ri (left eye). Projection distance, 67 cm. Fixation point to right of figure. Distance of *A* from fixation point, 30 cm. Distance *A B*, 9.8 cm. Distance *C D*, 15 cm. Distance *E F*, 10.5 cm. Distance *G H*, 13.5 cm.

large, the greater part of it would be unaffected by the presence of a blind spot. A rough preliminary investigation showed that the influence of the blind spot falls off rapidly as we recede from its edges.

A method of determining just how rapidly this falling off takes place was devised as follows. Small additions of Hering black paper were made to the original stimuli, first on one side of the blind spot and then on the other. Judgments of the length of the stimulus as seen were made by means of a comparison or measuring strip placed at the same angle of indirect vision as the standard, and parallel to it. Stated in greater detail, the method was as follows: A line was drawn on the screen parallel to the meridian on which the standard was located. On this line was placed a strip of Hering black paper, 1 cm. wide, and of a length which the observer judged equal to the combined length of the two standard stimuli. This first judgment varied for different observers and for different meridians from .5 cm. to 2 cm., according as the stimuli were placed on the exact limits of the blind spot or farther out in the field of vision. A strip of .3 cm. was then added to the original stimulus on the temporal side, making that strip .6 cm. in length. The stimulus now presented consisted of a strip of .3 cm. on the nasal edge of the blind spot, and one of .6 cm. on the temporal edge. This was compared in length with the measuring strip from the first experiment, and additions made to the measuring strip until it was again judged equal in length to the stimulus. The additions were continued alternately on the temporal and nasal sides of the stimulus until the comparison strip had attained a spatial value equal to the distance between the outer edges of the stimuli across the area of the blind spot. In the table recorded below, it will be seen that in the horizontal meridian the falling off in shrinkage is gradual, while in the other meridians, at times an increase of 10 cm. was required in the measuring strip when only .3 cm. was added to the stimulus. (See Table I.)

3. *The shrinkage in the visual field produced by the drawing together of its edges is compensated for by a magnification of spatial values in the region immediately surrounding the blind spot.* — The spatial values of visual sensations from the region immediately surrounding the blind spot seem to be a resultant of two factors: (*a*) the shrinkage influence due to the blind spot; and (*b*) the normal values of the neighboring portions of the retina removed from the influence of the blind spot. That is, the

shrinkage of the visual field due to the drawing together of its edges across the blind area is compensated for by a magnification or stretching of the spatial values of the region immediately surrounding it. Some such compensation should be expected, but that it should occur in so small an area is somewhat surprising.

As might be expected, this magnification is not equal along all meridians. It is obvious that it must sustain some relation to the breadth of the blind spot in the meridian considered and the width of the zone of spatial disturbance. That is, the amount of magnification per unit of surface should be directly proportional to the amount of shrinkage that has to be compensated for, and inversely proportional to the extent of area in which this compensation has to take place. The former factor is determined by the breadth of the blind spot; the latter by the breadth of the zone of spatial disturbance. Roughly speaking, the magnification should be directly proportional to the breadth of the blind spot in the meridian considered, and inversely proportional to the breadth of the zone of spatial disturbance.

An inspection of the drawings of the blind spot and the tables bears this out. We find (1) that the blind spot has its greatest breadth in the vertical meridian; a lesser breadth in the 45° meridians, and its least breadth in the horizontal meridian; (2) that the zone of spatial disturbance is broadest in the horizontal meridian, narrowest in the vertical meridian, and of mean breadth in the 45° meridians; and (3) that in accord with the principle of relation mentioned above, the greatest amount of spatial magnification is found to take place in the vertical meridian, a lesser amount in the 45° meridians, and the least amount in the horizontal meridian.

For example, in Table II it is found that for Observer C a stimulus in the vertical meridian which had an actual length of 1.7 cm. had an apparent length of 17.3 cm.; a stimulus in the 45° meridian (upper temporal, lower nasal) which had an actual length of 2.2 cm. had an apparent length of 14.8 cm.; a stimulus in the 45° meridian (upper nasal, lower temporal) with an actual length of 2.8 cm. had an apparent length of 16.2 cm.; and a stimulus in the horizontal meridian with an actual length of 3.7 cm. was found to have an apparent length of 14.8 cm. Similar results can be seen for Observer R.

4. *Distortion of form is produced when the borders of a figure fall within the region of shrinkage.* — Distortion of form is the direct corollary of

TABLE I.

TO SHOW FALLING OFF IN SHRINKAGE AS THE STIMULI RECEDE FROM THE EDGES OF THE BLIND SPOT. OBSERVERS C AND R.

Meridian.	Amount added to stimulus.		Side on which addition is made.	Distance between extremities of stimulus.			
				Observer C.		Observer R.	
	C.	R.		Actual	Apparent	Actual	Apparent.
	cm.	cm.		cm.	cm.	cm.	cm.
Horizontal	....	....	....	11.5	1.5	10.6	1.1
	.3	.1	Temporal	11.8	2.	10.7	1.3
	.3	.2	Nasal	12.1	4.8	10.9	1.7
	.3	.2	Temporal	12.4	6.	11.1	1.75
	.3	.2	Nasal	12.7	7.4	11.3	4.6
	.3	.2	Temporal	13.	9.5	11.5	5.5
	.3	.2	Nasal	13.3	10.8	11.7	7.
	.3	.2	Temporal	13.6	12.2	11.9	8.7
	.3	.2	Nasal	13.9	13.8	12.1	9.8
	.3	.2	Temporal	14.2	14.2	12.3	10.4
	....	.2	Nasal	....	....	12.5	11.
	....	.2	Temporal	....	....	12.7	12.1
	....	.2	Nasal	....	....	12.9	13.1
	....	...	....	16.	1.3	15.8	.8
	.3	.2	Upper	16.3	3.8	16.	1.

Vertical	.3	.2	Lower	16.6	9.8	16.2	1.5
	.3	.2	Upper	16.9	14.2	16.4	2.
	.3	.2	Lower	17.2	17.3	16.6	3.5
	....	.2	Upper	...	...	16.8	15.4
	....	.2	Lower	...	...	17.	18.4
Forty-fifth degree meridian, upper temporal, lower nasal.	...	...	....	13.2	1.5	15.5	.5
	.3	.3	Upper	13.5	2.3	15.8	1.
	.3	.3	Lower	13.8	7.8	16.1	1.5
	.3	.3	Upper	14.1	11.7	16.4	2.
	.3	.3	Lower	14.4	13.5	16.7	2.9
	.3	.3	Upper	14.7	14.8	17.	10.9
	...	.3	Lower	...	...	17.3	14.5
	...	.3	Upper	...	...	17.6	17.6
Forty-fifth degree meridian, upper nasal, lower temporal.	...	...	....	14.3	1.5	13.4	1.
	.3	.3	Upper	14.6	2.	13.7	1.5
	.3	.3	Lower	14.9	3.2	14.	2.2
	.3	.3	Upper	15.2	4.5	14.3	3.3
	.3	.3	Lower	15.5	12.2	14.6	7.1
	.3	.3	Upper	15.8	15.1	14.9	12.3
	.3	.3	Lower	16.1	16.2	15.2	15.2

TABLE II.

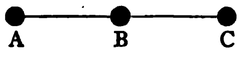
To show Compensation for Shrinkage by Magnification of Spatial Values in the Region immediately surrounding the Blind Spot. Observers C and R.

Observer C.			Observer R.		
Meridian.	Length of stimulus.		Meridian.	Length of stimulus.	
	Actual.	Apparent.		Actual.	Apparent.
Horizontal. (Breadth of blind spot, 10.5 cm. Distance between extremities of stimuli, 11.5 cm. Total initial length of stimuli outside blind spot, 1 cm.)	cm. 1.	cm. 1.5	Horizontal. (Breadth of blind spot, 10.3 cm. Distance between extremities of stimuli, 10.6 cm. Total initial length of stimuli outside blind spot, .3 cm.)	cm. .3	cm. 1.1
	1.3	2.		.4	1.3
	1.6	4.8		.6	1.7
	1.9	6.		.8	1.75
	2.2	7.4		1.	4.6
	2.5	9.5		1.2	5.5
	2.8	10.8		1.4	7.
	3.1	12.2		1.6	8.7
	3.4	13.8		1.8	9.8
	3.7	14.8		2.	10.4
Vertical. (Breadth of blind spot, 15.5 cm. Distance between extremities of stimuli, 16 cm. Total initial length of stimuli outside blind spot, .5 cm.)	.5	1.3		2.2	11.
	.8	3.8		2.4	12.1
	1.1	9.8		2.6	13.1
	1.4	14.2	Vertical. (Breadth of blind	.2	.8
	1.7	17.3		.4	1.

Forty-fifth degree meridian, upper temporal, lower nasal. (Breadth of blind spot, 12.5 cm. Distance between extremities of stimuli, 13.2 cm. Total initial length of stimuli outside blind spot, .7 cm.)	.7	1.5	spot, 15.6 cm. Distance between extremities of stimuli, 15.8 cm. Total length of stimuli outside blind spot, .2 cm.)	.6	1.5
	1.	2.3		.8	2.
	1.3	7.8		1.	3.5
	1.6	11.7		1.2	15.4
	1.9	13.5		1.4	18.4
	2.2	14.8	Forty-fifth degree meridian, upper temporal, lower nasal. (Breadth of blind spot, 15.3 cm. Distance between extremities of stimuli, 15.5 cm. Total initial length of stimuli outside blind spot, .2 cm.)	.2	.5
				.5	1.
				.8	1.5
....	....	....		1.1	2.
				1.4	2.9
				1.7	10.9
....	....	....		2.	14.5
				2.3	17.6
Forty-fifth degree meridian, upper nasal, lower temporal. (Breadth of blind spot, 13.3 cm. Distance between extremities of stimuli, 10.6 cm. Total initial length of stimuli outside blind spot, .3 cm.)	1.	1.5	Forty-fifth degree meridian, upper nasal, lower temporal. (Breadth of blind spot, 12.7 cm. Distance between extremities of stimuli, 13.4 cm. Total initial length of stimuli outside blind spot, .7 cm.)	.7	1.
	1.3	2.		1.	1.5
	1.6	3.2		1.3	2.2
	1.9	4.5		1.6	3.3
	2.2	12.2		1.9	7.1
	2.5	15.1		2.2	12.3
	2.8	16.2		2.5	15.2

shrinkage. It has been reported by Wittich,[24] Aubert,[25] Funke,[26] and Bull,[27] who did not attribute it to shrinkage; and denied by Weber,[28] Volkmann,[29] Fick and DuBois-Reymond,[30] Helmholtz,[31] and others, who all used their results as an argument against shrinkage.

Since shrinkage takes place over but a narrow area around the blind spot, distortion of form can be expected to occur only when the margins of a figure fall within this area.

In most cases in the work of former experimenters, the relative breadth of figure and blind spot cannot be determined. It can only be inferred that the margins of the figures fall outside this probable zone of disturbance from statements that are given as to the size of the figures and the distances to which they are removed from the eye. Three cases have been found by us, however, in which definite information is given as to the relative size of the blind spot and of the figures used. Only one of these refers to distortion of form. The other two refer to shrinkage. All three will, however, be considered at this point. In all, the distortion or shrinkage, as the case happened to be, took place when the margins of the figure fell within the zone of disturbance, and did not take place when the margins fell outside of this zone. The three cases are as follows. The first is an observation described by Volkmann in 1840[32] to illustrate his theory of local signs. Using the figure  he caused all the points lying between *A* and *C* to fall upon the blind spot. *A* and *C* thus fell upon the margins of the blind spot, and according to our results, shrinkage should have been expected. The shrinkage was obtained. The line *AC* appeared very much shortened. He offers the following explanation for the shortening. The presence of two discrete sensitive points on the retina furnishes the mind with a visual unit of measure. A visual object is, then, as many times this unit in size, as the part of the retina stimulated by the image of the object contains discrete sensitive points. In the above experiment the line *ABC* falls upon very few sensitive points, and is, therefore, seen as very short.

[24] Wittich: Archiv für Ophthalmologie, 1863, ix$_3$, p. 1.

[25] Aubert: Physiologie der Netzhaut, 1865, p. 256.

[26] Funke: Freiburg Berichte, 1866, iii$_3$, pp. 1–15.

[27] Bull: Perimetrie, 1895, p. 8.

[28] Weber: *Op. cit.*, pp. 153–159.

[29] Volkmann: *Op. cit.*, pp. 27–50.

[30] Fick and DuBois-Reymond: *Op. cit.*, pp. 390–407.

[31] Helmholtz: *Op. cit.*, pp. 717–727.

[32] Volkmann: Wagner's Handwörterbuch, 1846, iii, p. 337.

The second also is an observation made by Volkmann.[23] It was described in 1853 in support of his doctrine of associative filling in. In this experiment the margins of his figure did not fall within the zone of disturbance, as this zone is estimated by us, and shrinkage did not take place. The following figure was used: *A*———*B* *C*———*D*. When points *B* and *C* were made to fall just within the blind spot, a continuous line *AD* was seen, whose length was equal to the total distance actually existing between *A* and *D*. Volkmann explains this result as follows: The continuity of the line *AD* is due to associative filling in. But according to his theory of local signs, this filling in does not prevent the line *AD* from becoming shorter, for the apparent distance between *A* and *D* is determined solely by the number of retinal points stimulated. The distance *AD* should, then, have been shortened just as the distance *AC* was shortened in the first figure. In order to explain this apparent contradiction in his results, he supplements his theory to include an interaction between retinal points. He argues that the points above and below the level of the blind spot, lying in the line running through *A* perpendicular to *AD*, prevent by their influence upon space perception the point *A* from being drawn towards the point *D*. Similarly the points lying in the line running through *D* perpendicular to *AD* prevent *D* from being drawn towards *A*. This explanation is obviously unsatisfactory. It offers no explanation why *A* and *C* of the first figure are not also prevented from being drawn together by the influence of the points lying in the lines running through *A* and *C* perpendicular to *AC*. The two cases are, however, easily understood in terms of our results. In the first case, the points *A* and *C* lie within the zone of shrinkage and are drawn towards each other. The line *AC* is thus shortened. In the second case, the continuity of the line *AD* is explained by the drawing together of the points *B* and *C*. The line *AD* is not shortened, however, by the drawing together of *B* and *C* because the points *A* and *D* lie well outside the zone of disturbance, and are, therefore, not affected by the blind spot. Further, the shrinkage caused by the drawing together of the points *B* and *C* is compensated for by the magnification of space values in the region immediately surrounding the blind spot. The line *AD* thus appears unbroken and of full length. The magnification of space values is not a new concept. It is well known that there is considerable variation of space values in different parts of the peripheral retina. The zone around the blind spot may be considered merely as an exceptional and extreme case due to conditions just as extreme and exceptional. No explanation will be attempted here of the magnification. It becomes a problem for explanation along with the other cases of variations in space values.

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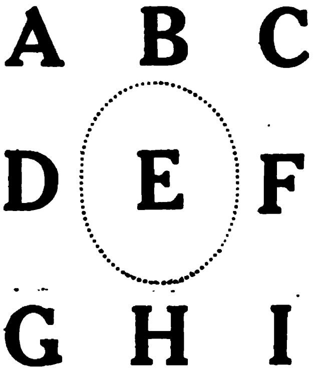
[23] VOLKMANN: Berichte über die Verhandlungen der königlich-sächsischen Gesellschaft der Wissenschaften zu Leipzig, mathematisch-physische Classe, 1853, p. 37.

The third case in point is an observation made by Helmholtz[34] to demonstrate the absence of distortion of form. An exact copy of the figure used by Helmholtz is given below.



The dotted line around the letter *E* represents the exact size of his blind spot projected on a card 30 cm. distant. This area is 2 cm. in width. The breadth of the zone around it in which shrinkage should take place according to our determinations would at most not exceed .3 cm. Within this zone lies only white background. The letters *B*, *D*, *F*, and *H*, then, are all practically outside the area of this zone and should not be influenced by it. Hence, granting shrinkage, there is no reason why the positions of *B*, *D*, *F*, and *H* should be disturbed because of their spatial relation to the blind spot. Helmholtz copied this figure from Volkmann. In the figure published by Volkmann,[35] the outline of the observer's blind spot is not shown. It is to be regretted that we cannot, therefore, determine in the case of the original experiment whether or not, according to our results, distortion should have been expected.

Two devices were used by us to demonstrate distortion. In the first, two strips .3 cm. wide and 30 cm. long were fastened on the screen parallel to each other and tangent at their centres to opposite edges of the blind spot. The usual precautions were observed to guarantee that each was without the blind area. In the second, the blind spot was outlined by a narrow margin of black paper. In the first case,

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[34] Helmholtz: *Op. cit.*, p. 723.

[35] Volkmann: *Op. cit.*, p. 35.

when the point was fixated, the strips were seen parallel in their upper and lower portions, but for a short distance near the centre they appeared to be discontinuous, and a single narrow black figure of somewhat indefinite outline was seen to occupy a position somewhere between the lines of direction of the upper and lower segments of the strips. In the second case, when the point was fixated, the observer saw a continuous but very shrunken black figure of indefinite shape. Sometimes, but rarely, it was seen shrunken in all directions. Usually it was seen drawn together in only one dimension at a time, now in the horizontal and now in the vertical. For one observer the figures thus produced alternated during a single prolonged observation. In this case we have a different kind of shrinkage from that presented by two stimuli on opposite edges of the blind spot. We could expect in the former instance complete shrinkage in all directions at once only if the blind spot were circular in form, in which case every point on its edges would be separated from every other point by an insensitive area. When, however, the figure is very much elongated and its outline in places approximates a straight line, complete shrinkage can take place only between points on opposite margins and not between points on the same side of the figure; that is, the figure could not be expected to be seen at any one time completely shrunken in all directions, but, as was reported, namely, a figure shrunken now in one direction, now in another.

5. *There is no associative filling in of the blind spot, but a drawing together of its edges.* — Our work gives no evidence of any trace of associative filling in. The drawing together in sensation of opposite edges of the blind spot is complete. When narrow stimuli were placed on opposite edges of the blind area, they were invariably seen as one, occupying an intermediate position of somewhat indefinite location. When additions were made to these stimuli giving in sensation the rapid increase in spatial dimensions recorded in the tables, the stimulus was still seen as continuous. Similar results were obtained in the case of the outline figure described in the preceding chapter. The figure produced by this shrinkage, whatever its shape, was always seen as continuous.

Before leaving this topic, account should be taken of a few exceptional observations made by R. When testing the falling off in shrinkage, as we recede from the edges of the blind spot, after additions had been made to the original stimulus, this observer, at the moment of exposure, always reported a continuous strip; but in a few cases, after long exposure, she reported two strips separated by a narrow white space. There are two possible explanations of this variation. The distance between the strips may have been filled in associatively by the background, or the white sensation may have been caused by an eye movement which allowed the actual white in the area between the stimuli to be sensed. The latter alternative seems the more probable, as but three or four instances of the presence of white were reported. To test this, however, a model of the blind spot was cut from Hering black paper and fastened to the screen between the stimuli. Under these conditions, white was never seen in the area occupied by the blind spot, although there was just as much chance for associative filling in with white as there was in the former observation. This indicates that the white noticed in the former experiments was the actual white existing between the stimuli, as seen by eye movement.

CONCLUSION.

Our experiments, then, yield the following results:

1. Complete shrinkage takes place when the stimuli observed are on opposite edges of the blind spot. Opposite edges of the blind spot function as contiguous retinal areas.

2. There is a rapid falling off of the influence of the blind spot upon spatial values as the stimuli recede from its edges. This zone of disturbance averages for all meridians less than one-sixth the breadth of the blind spot.

3. The shrinkage in the visual field produced by the drawing together of its edges across the blind area is compensated for by a magnification of spatial values in the region immediately surrounding the blind spot. In proportion to the breadth of the zone of disturbance, this magnification is greater or less per unit area of stimulus in different meridians. It is about twice as great in the vertical as in the horizontal meridian and about one and one-half times as great in the 45° meridian.

These results serve the following purposes: (*a*) They offer an explanation of the absence of a gap in the monocular field of vision, without recourse to the wholly speculative doctrine of associative filling in. (*b*) They destroy the experimental basis for its use as a general principle of explanation in other sense fields. (*c*) They indicate why Helmholtz and other observers have failed to find shrinkage. Figures whose margins extended beyond the zone of shrinkage were used, and judgments made as to decrease in size or distortion in form. No effect could be noticed, and none should have been expected, as our results show. (*d*) They make some slight contribution to the subject of visual space perception. For example, the widely different spatial values in the zone of disturbance for the horizontal, vertical, and 45° meridians could scarcely be explained in terms of existing empiristic theories.

The analogue of this problem is now being worked out by one of the writers (Ferree) in skin sensation. Recourse has been had by various systematists to the doctrine of associative filling in to explain why the skin should give a continuum of sensation in case of a real stimulation. So far in the work the results are wholly against such a principle of explanation.

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STUDIES IN EXPERIMENTAL GLYCOSURIA. — VIII. THE RELATIONSHIP OF THE ADRENAL GLANDS TO SUGAR PRODUCTION BY THE LIVER.

By J. J. R. MACLEOD and R. G. PEARCE.

[*From the Physiological Laboratory, Western Reserve University.*]

HAVING shown that even the most extreme variations in the amount of sugar produced by the liver are unaccompanied by any change in the glycogenolytic activities of extracts of this organ prepared in various ways,[1] it became necessary, as our next step, to study the influence of different substances in the blood on hepatic glycogenolysis. Granted that the hydrolysis of glycogen to dextrose is dependent upon the relatively small and constant supply of glycogenase which the liver contains, we must assume that the variable degrees of glycogenolysis which the intact liver exhibits are due either to a change in the condition of the glycogen or to the presence in the blood of substances which can accelerate or retard the action of the glycogenase. By changes in the condition of the glycogen, we refer to the manner of its deposition and fixation in the hepatic cell. Little is known regarding this question, but it is possible that the glycogen may not be merely deposited in masses in the liver cell, but be fixed in some form of combination with a sustentacular substance (*Träger-Substanz*).[2] So long as it is thus combined the glycogen will be protected from the action of glycogenase, but become immediately attacked by this ferment when it is set free. This fixation of glycogen may be influenced by the presence of hormones or it may be under nerve control. The hormone control need not, however, be focussed on the fixation of glycogen, but it may act as an accelerator or inhibitor of glycogenase. The glycogenase present in the liver may, in other words, depend for its activation upon substances present in the blood, and the degree of its activity be thus proportional to the amount of these substances. It may be the activating substances and not the amount of glycogenase, or the condition of the glycogen, that is under nerve control.

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[1] MACLEOD and PEARCE: this Journal, 1911, xxviii, p. 403.

[2] ARNOLD: Virchow's Archiv für pathologische Anatomie, 1908, cxciii, p. 174.

We have prefaced our present paper with these theoretical considerations in order to make plain the reason for the research of which it reports the results. We had to seek for substances in the blood that could accelerate hepatic glycogenolysis. We know that glycogenase, like other diastatic enzymes, is very susceptible to the H-ion concentration of its environment, a slight increase accelerating the process. It has been stated that post-mortem glycogenolysis is largely due to acceleration by the acid substances produced by the autolytic process that follows death, but it is improbable, in the light of L. J. Henderson's conclusions,[3] that any such mechanism could play a rôle in the living animal.[4]

Excess of the sodium ion in the blood causes glycosuria,[5] possibly because it accelerates the activity of glycogenase, but this process — if indeed the sodium ion does have such an influence — cannot be of any importance in the normal control. On the other hand, during recent years there has been amassed much evidence indicating a close association between certain ductless glands and sugar metabolism. The parathyroids, the adrenals, the pancreas, and the pituitary gland have undoubtedly some function in this direction. We must assume that it is the glycogenolytic process upon which the internal secretions of these glands act. There is at least, so far, nothing to indicate that the glycolytic process undergoes variations in the animal body independent of accompanying changes in the glycogenolytic. Be that as it may, it is obvious that, as our next step, it became necessary to make a careful study of the relationship of the various ductless glands to sugar production by the liver. The present communication contains results which indicate the rôle of the adrenal gland in this connection.

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[3] Henderson, L. J.: this Journal, 1906, xv, pp. 257–271; also Henderson and Black: *Ibid.*, 1907, xviii, p. 250.

[4] Pavy and Bywaters: Journal of physiology, 1910, xli, p. 168.

[5] Fischer, M.: Archiv für die gesammte Physiologie, 1904, cvi, p. 80; Brown, O. H.: this Journal, 1904, x, p. 378.

There is already some evidence that the adrenals are of importance in the regulation of the glycogenolytic function. Mayer[6] found in rabbits that after double adrenalectomy piqure failed to produce glycosuria, and the same was found in three dogs that survived the adrenalectomy. Pancreatectomy along with adrenalectomy in dogs is too severe an operation to yield satisfactory results. In six cases, however, removal of the left adrenal (the right having previously been removed) in eight days after pancreatectomy caused a distinct decrease in glycosuria in the short time (two to five hours) during which the animal survived this last operation.[7] Similar results have been obtained by Frouin.[8]

It has been stated that, after adrenalectomy, stimulation of the splanchnic nerves does not produce glycosuria.[9] Porges[10] found a progressive diminution in the blood sugar to occur after adrenalectomy in dogs; and in patients with Addison's disease this same author describes hypoglycæmia as being present.

It is unnecessary, in this place, to review the literature bearing on the actual secretion of adrenalin from the adrenal gland into the blood. That this secretion is under control of the sympathetic system, that is to say, is increased by stimulation of the corresponding splanchnic nerve, has been demonstrated by Asher, Popielski, Tscheboksareff, etc.[11] It is of course well known that injection of the active principle of the adrenal (adrenalin) causes hyperglycæmia and glycosuria, and it would appear that the glycogen content of the liver is an important factor in the intensity of the hyperglycæmia, at least in the initial injection, although, as Pollak[12] has shown, it still has an action on carbohydrate metabolism when, prior to its injection, there is no glycogen present in the liver.

[6] MAYER: Comptes rendus de la Société de Biologie, 1908, lx, p. 1124.

[7] MAYER: *Ibid.*, 1910, lxiv, p. 219.

[8] FROUIN: *Ibid.*, 1910, lxiv, p. 219.

[9] GAUTRELET and THOMAS: *Ibid.*, 1909, lxvii, p. 233.

[10] PORGES: Munchener medicinische Wochenschrift, lvi, p. 156.

[11] ASHER: Zentralblatt für Physiologie, 1910, p. 927; TSCHEBOKSAREFF: *Ibid.*, 1910, cxxxvii, p. 137; POPIELSKI: Archiv für die gesammte Physiologie, 1911, cxxxix, p. 571.

[12] POLLAK: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, p. 166.

METHODS.

The experiments in the present research were performed on etherized dogs, fed, on the day before the experiment, with sugar, bread, and flesh. In a few of the livers of these animals, which we examined for glycogen, large quantities of this substance were found present. Throughout the experiments the body temperature was kept constant by means of a hot tank.

A long glass cannula was inserted through the renal vein so that its open end lay in the inferior vena cava opposite the hepatic veins. The samples of blood for analysis were aspirated through this cannula with the precautions fully described in our previous communication.[13] In the majority of the earlier experiments the reducing substance in the blood was estimated by the method of Waymouth Reid. In most of the later experiments, on the other hand, the aspirated blood was mixed with oxalate solution, centrifuged, and the plasma used for analysis by the Rona-Michaelis method.[14] In some of the experiments both methods were employed. The reducing power of the protein-free blood extracts was determined by the method of Bang.

RESULTS.

1. *Stimulation of the left splanchnic nerve after removal of the left adrenal gland.* — The initial observations in the present research consisted in comparing the immediate effect on the percentage-reducing power of the blood (removed as above described) produced by stimulation of the splanchnic nerve on the left side, before and after removal of the adrenal gland. Although we have already published these results, it will be well, for the sake of comparison, to restate them along with some others here. This is done in Table I.[15]

Clearly, the removal of the left adrenal gland renders stimulation of the left splanchnic nerve incapable of producing hyperglycæmia within

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[13] MACLEOD and PEARCE: this Journal, 1911, xxviii, p. 403.

[14] *Cf.* OPPLER and RONA: Biochemische Zeitschrift, 1908, xiii, p. 121.

[15] A preliminary communication of these results was made before the Society of Experimental Biology and Medicine in April, 1911. *Cf.* Proceedings of the Society of Experimental Biology and Medicine, 1911, viii, p. 110.

TABLE I.

THE EFFECT ON THE AMOUNT OF REDUCING SUBSTANCE IN THE BLOOD OR BLOOD PLASMA OF THE INFERIOR VENA CAVA PRODUCED BY STIMULATION OF THE LEFT SPLANCHNIC NERVE.

No. of expt.	Blood or plasma.	Per cent reducing substance.		Change produced. (Figs. = per cent rise.)	Remarks.
		Before stimulation.	After stimulat'n.		
A. WITH ADRENALS INTACT.					
16	Blood	0.136	0.176 (*7*)[1]	29	
18	"	0.124	0.110 (*7*)	Fall	No rise in blood pressure on stimulating nerve.
19	"	0.130	0.176 (*10*)	35	
20	"	0.199	0.265 (*5*)	33	
21	"	0.151	0.195 (*5*)	28	
24	"	0.132 0.121 (30 min. later)	0.154 (*7*)	27	
25	"	0.194 0.213 (30 min. later)	0.280 (*5*)	31	
32	"	0.111 0.122 (30 min. later)	0.166 (*9*)	36	
90	Plasma	0.180	0.254 (*8*)	41	Bile duct and hepatic artery ligated.
79	"	0.216	0.238 (*9*)	10	
B. WITH LEFT ADRENAL REMOVED.					
27	Blood	0.118	0.124 (*10*) 0.109 (*25*)	..	
28	"	0.120 0.082 (30 min. later)	0.101 (*5*) 0.103 (*20*)	..	
29	"	0.171 0.173 (30 min. later)	0.177 (*16*)	..	
30	"	0.163 0.127 (30 min. later)	0.113 (*15*)	..	
31	"	0.249 0.248 (25 min. later)	0.270 (*5*) 0.256 (*20*)	..	Blood examined by Rona-Michaelis method. Blood pressure low throughout.
49	"	0.242	0.225 (*12*)	..	

[1] The figures in Italic indicate the number of minutes elapsing between the removal of the various samples of blood. In order, the figures are to be read from left to right.

the period of time (less than ten minutes) chosen in the observations. Several explanations for this result are possible:

1. That the underlying suprarenal plexus was mechanically damaged by the operative procedure.
2. That the hyperglycogenolysis which ordinarily follows splanchnic stimulation is due to hypersecretion of adrenalin into the blood.

Regarding the former possibility, we cannot be certain that glycogenolytic fibres were not damaged. It is a well-known anatomical fact that the medulla of the adrenal gland is developed from the same embryonic tissue as the sympathetic system, and that many nerve fibres of the underlying suprarenal plexus are pulled in, as it were, into the adrenal medulla. Bearing these facts in mind, we have been careful to do as little damage to the underlying plexus as possible; we have always convinced ourselves by post-mortem dissection that the main branches of the plexus were intact. Besides, we have experimental evidence that the vasomotor fibres, at least, were not seriously damaged, for the usual rise in arterial blood pressure was obtained when the nerve was stimulated in all the recorded experiments. But yet the possibility remains that the glycogenolytic fibres had suffered injury.

Although, in from ten to twenty-five minutes after the application of the stimulus, no hyperglycæmia was noted, it is possible that with more prolonged stimulation a certain degree of hyperglycæmia might be obtained. We have not as yet investigated this possibility.

Stimulation of the left splanchnic nerve after ligation of the adrenal veins. — In order to avoid injury to the underlying nerves, ligatures were tied around the adrenal veins, after which the splanchnic nerve on the left side was stimulated. In three experiments of this nature (see Table II, Nos. 80, 82, and 84) a distinct hyperglycæmia occurred in one, whereas in the other two this was not the case until towards the end of the experiment, when other factors, such as low blood pressure, probably account for the result.[16] In one experiment (No. 56) the vein was ligated only on the left side, and it was found that splanchnic stimulation caused hyperglycæmia.

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[16] We have found that when there is a low blood pressure (below 60–70 mm. Hg) the blood of the vena cava almost always shows a progressive increase in reducing substance, which of course renders the results of nerve stimulation, etc., inconclusive.

In the remaining experiment of this series (No. 79) splanchnic stimulation caused hyperglycæmia prior to ligation of the adrenal veins, but not so after it. In the former case, however, the plasma alone was examined for reducing power; in the latter, the whole blood.

TABLE II.

THE EFFECT ON THE AMOUNT OF REDUCING SUBSTANCE OF THE BLOOD PLASMA OF THE VENA CAVA INFERIOR PRODUCED BY STIMULATION OF THE LEFT SPLANCHNIC NERVE, AFTER LIGATION OF THE ADRENAL VEINS.

No. of expt.	Per cent reducing substance.		Change produced. (Figs. = percentile rise.)	Remarks.
	Before stimulation.	After stimulation.		
80	0.199	0.167 (*10*)[1]	Fall	Coil, 6 cm.
	0.173 (*20*)	0.199 (*10*)	15	
	0.183 (*15*)	0.241 (*10*)	30	Blood pressure, 60 mm. Hg.[2]
82	0.266	0.249 (*8*)	Fall	Marked hyperpnœa throughout experiment. Coil, 6 cm.
	0.300 (*23*)	0.255 (*8*)	"	
	0.317 (*13*)	0.321 (*5*)	No change	Blood pressure very low.
84	0.177	0.168 (*6*)	Fall	Coil, 6 cm.
	0.138 (*12*)	0.110 (*7*)	"	
	0.120 (*20*)	0.140 (*7*)	16	Blood pressure, 60 mm. Hg. Coil, 4 cm.
56	0.200	0.225 (*8*)	12.5	The right adrenal vein was not ligated.
	....	0.268 (*28*)		
79	0.216	0.238 (*9*)	10	Coil, 6 cm. Adrenal veins intact.
Combined expt.	0.202[3]	0.196 (*8*)[3]	Fall	Adrenal veins ligated.

[1] The figures in Italic indicate the number of minutes elapsing between the removal of the various samples of blood. In order, the figures are to be read from left to right.

[2] Unless otherwise noted, the arterial blood pressure was between 90–120 mm. Hg.

[3] Blood used for analysis.

On account of the uncertainty of these results we did not continue the experiments. It is possible that, in some cases at least, there may be small venules which are left unligated and through which the adrenalin gains the circulation.

Stimulation of the splanchnic with the adrenal gland intact, but the hepatic nerves cut: — There is, so far, nothing, either in our own results or in those of previous investigators, to justify the conclusion that it is on account of a secretion of adrenalin into the blood that the hyperglycogenolysis following nerve stimulation is due. Were this the case, then we should find:

1. That after complete section of the nerve paths between the adrenal gland and the liver, both adrenals being intact, stimulation of the splanchnic nerve would produce the same degree of hyperglycæmia as is obtained with the nerves uncut.

The results of such experiments are recorded in Table III. In the earlier experiments (Nos. 45 to 54) the operative procedure consisted in cutting all the structures running to the liver (after double ligation) except the portal vein. It is only in this way that one can be certain that all the fibres of the hepatic plexus are cut. The ligation of the hepatic artery and bile ducts involved in this operation does not within the time of the experiment introduce any disturbing factors, for when these two structures are ligated, but the veins left intact, stimulation of the splanchnic nerve is followed by the usual hyperglycæmia. This is seen in the results of the experiment numbered 90 on Table I. It has been stated that ligation of the ducts does ultimately lead to depletion of the liver glycogen; but this is evidently too slow a process of glycogenolysis to effect the reducing power of the blood in the vena cava. That mere ligation of the hepatic artery produces no change in blood sugar was shown by one of us some years since.[17]

In the first four experiments recorded in the table inconstant results were obtained. In one experiment (No. 54) an undoubted rise followed the splanchnic stimulation; in another (No. 45) a rise was noted when the blood removed after stimulation was compared with that removed immediately preceding it, although when compared with the blood removed at the beginning of the experiment no rise was apparent. In he remaining two experiments (Nos. 46 and 53) the splanchnic stimulation did not produce any definite effect on the reducing substance in the blood. The experiments were therefore repeated with the difference that, besides cutting all the structures of the hepatic pedicle save the portal vein, the outer coat of this latter was cauterized so as

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[17] Macleod: this Journal, 1900, xxii, p. 373.

TABLE III.

The Effect on the Reducing Substance of the Blood or Blood Plasma of the Vena Cava Inferior produced by Stimulation of the Splanchnic Nerves after cutting the Hepatic Plexus.

No. of expt.	Per cent reducing substance in blood.			Per cent reducing substance in plasma.			Remarks.
	Before stimulation.	After stimulation.	Change produced.	Before stimulation.	After stimulation.	Change produced.	
45	0.172	......	......	......	......	Nil	Reid method for bloods.
	(0.136 (*25*))	0.168 (*8*)	?	......	......	......	
46	......	......	......	0.255	0.245 (*8*)	Nil	
53	0.116	0.123 (*6*)	6% rise	0.142	0.100 (*6*)	Fall	Reid method for bloods.
	......	0.086 (*36*)	......	......	0.118 (*36*)	......	
54	0.255 (R)	0.360 (*11*)	41% rise	0.163	0.243 (*11*)	49% rise	R=Reid method.
	0.219 (M)	......	......	......	0.290 (*41*)	......	M=Michaelis method.
65	0.130	......	......	0.161	......	......	Vein seared. Rona-Michaelis method for blood.
	0.116 (*14*)	0.1295 (*10*)	11.6% rise	0.125 (*14*)	0.166 (*10*)	33% rise	
66	......	......	......	0.241	0.259 (*16*)	......	Vein seared.
	......	......	......	......	0.280 (*28*)	7% rise	
68	0.188	0.182 (*6*)	Fall	......	......	......	Vein seared.
	0.179 (*20*)	0.181 (*5*)	No change	......	......	......	
75	......	......	......	0.254	0.268 (*3*)	5.5% rise	Vein seared.
	......	......	......	0.232 (*20*)	0.254 (*3*)	9.4% rise	
76	......	......	......	0.129	0.134 (*6*)	3.8% rise	Vein seared.
	......	......	......	0.156	0.154 (*5*)	No change	

to be certain that every nerve fibre was severed. The cauterization was produced by means of a red-hot wire applied for a moment to the vein at various places so as to produce linear sears across the vein, these being arranged so as to interdigitate with one another. In the five experiments in which this was done (Nos. 65, 66, 68, 75, and 76) no splanchnic hyperglycæmia occurred in two (increases of less than 5 per cent are discounted) and in two others it was moderate, the increase in reducing power being below 10 per cent. In the remaining experiment of this group (No. 65) a definite hyperglycæmia resulted from the stimulation. It can certainly be concluded that the rupture of the nerve path has at least greatly minimized the intensity of the hyperglycogenolysis which otherwise follows stimulation of the great splanchnic nerve. As a corollary it follows that it cannot be solely because of a hypersecretion of adrenalin into the blood that stimulation of the splanchnic nerve causes hyperglycogenolysis. The increased adrenalin content in the blood may be a contributory factor to the hyperglycogenolysis, but it is not everything. The failure of the increased adrenalin content of the blood, produced by splanchnic stimulation, to stimulate hyperglycogenolysis by itself merely indicates that the amount is insufficient. It is of course possible that were the stimulation in these experiments kept up for a longer period of time enough adrenalin would accumulate in the blood to excite the hyperglycogenolysis. Moreover, it must not be lost sight of that with the hepatic arteries ligated the adrenalin must circulate through the intestinal capillaries before gaining the liver. We do not know what may happen to adrenalin in the intestinal walls. That a considerable destruction of it occurs here, has been shown by Elliott.[18] Unfortunately, for want of time we are at present unable to make any special investigation of this question.

Injection of adrenalin in portal vein after cutting hepatic nerves. — The above results prompt the question as to the exact structure, concerned in the glycogenolytic process, upon which the adrenalin acts. Does it act on the glycogenic function of the liver directly, or does it act on some part of the nerve path that controls this function? Although improbable, it might yet be that adrenalin does not directly act on the glycogenolytic nerve terminations in the hepatic cells, but on their fibres, say in the cœliac plexus. In such a case section of the hepatic plexus would render adrenalin injections incapable of producing hyperglycæmia.

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[18] Elliott: Journal of physiology, 1905, xxxii, p. 401.

The results given in Table IV show that adrenalin acts directly on the peripheral mechanism in the liver. In the two experiments recorded, all of the structures to the liver except the portal vein were cut and the outer coat of the vein cauterized, in the manner already described. Through a cannula inserted in the pancreatico-duodenal vein 12 c.c. of either 1 in 5000 adrenalin chloride solution or of a similar amount of Locke's solution was injected, a sample of blood for analysis

TABLE IV

The Effect on the Reducing Power of the Blood of the Vena Cava Inferior produced by Injection in the Portal Vein of 12 c.c. 1–5000 Adrenalin Chloride or an Equal Volume of Locke's Solution. Hepatic Nerves cut.

No. of expt.	Time in minutes since removal of first blood.	Solution injected.	Per cent. reducing substance.	Percentile rise.	Remarks.
69	0	......	0.242	..	The samples of blood were removed immediately after injection completed, except in fourth blood. The figures in each case are the average of closely agreeing duplicates.
	10	Locke sol'n	0.257	6	
	17	Adrenalin	0.272	58	
	20	......	0.315	22	
	30	Locke sol'n	0.226	....	
	35	Adrenalin	0.343	52	
73	0	......	0.168	..	Because of clotting the last four bloods were measured in cylinders and the duplicates were not so close as usual.
	25	Locke sol'n	0.140	..	
	5	Adrenalin	0.180	28	
	20	No injection	0.221	11	
	23	Locke sol'n	0.228	3	
	26	Adrenalin	0.263	15	
	50	Locke sol'n	0.190	..	
	54	Adrenalin [1]	0.263	39.5	

[1] Adrenalin injection had no effect on blood pressure.

being shortly after aspirated from the vena cava and examined for reducing power. A very striking increase in reducing power was observed in every instance in which adrenalin was injected. Even when the blood sample was removed simultaneously with the injection, the increase could be noted. We are aware that these amounts of adrenalin are very large. It would be of interest to determine the minimal dose which could cause hyperglycogenolysis when injected as above, and compare it with the minimal dose necessary when the adrenalin is injected in the systemic circulation. However, for our present purpose the results are sufficient; they indicate that adrenalin directly stimulates hepatic glycogenolysis. This being the case, we must conclude that there is not a sufficient hypersecretion of adrenalin from the adrenal gland into the blood as a result of stimulation of the splanchnic nerve to directly excite hepatic glycogenolysis. Even at the risk of repetition, we must point out that in the experiments recorded in Table II the hepatic arteries were occluded; had they been patent, the adrenalin produced by hypersecretion of the adrenal gland might have produced hyperglycogenolysis. We cannot, however, be certain of cutting all branches of the hepatic plexus without cutting the hepatic arteries.

Stimulation of the hepatic nerves without removal of the adrenal glands.— We have attacked the problem from another point of view, namely, by investigating the effect on hepatic glycogenolysis of stimulation of the hepatic plexus, before and after removal of the adrenal glands. With the adrenal glands intact, stimulation of the hepatic plexus, either cut or uncut, causes a very marked rise in the reducing power of the blood plasma of the vena cava (see Table V). In three minutes after the application of the stimulus the increase amounted to 4 per cent, and from this up to eight minutes it became progressively more marked. In one case with a very strong stimulus (secondary coil, 4 cm.) the increase in six minutes amounted to 94 per cent. This result is, however, perhaps an exaggerated one an account of the blood pressure being low.

It is of importance, as showing the close relationship between the nerve stimulation and the increased amount of reducing substance in the blood, to note that there occurred in one of the experiments (No. 81) a marked decrease in the reducing substance during the interval which followed the removal of the stimulus. This interval was usually fifteen to twenty minutes, and is indicated by the figures in brackets placed after the results. This decline between the periods of stimulation is not seen in Experiment 83, probably on account of the fact that a very strong stimulus (secondary coil, 4 cm.) was employed.

TABLE V.

THE EFFECT ON THE REDUCING SUBSTANCE OF THE BLOOD PLASMA OF THE VENA CAVA INFERIOR PRODUCED BY STIMULATION OF THE HEPATIC NERVES WITH THE ADRENALS INTACT.

No. of expt.	Per cent reducing substance.		Change produced. (Figs. = per cent increase.)	Remarks.
	Before stimulation.	After stimulation.		
81	0.153	0.179 (*6*)[1]	17	Coil, 6 cm. Pedicle uncut. Rise in B. P. following stim.
	0.165 (*17*)	0.204 (*7*)	23.6	Pedicle cut. Rise in B. P. now very slight. Coil, 4 cm.
	0.150(*16*)	0.294(*6*)	94	
83	0.125	0.160 (*8*)	20	Coil, 4 cm. Pedicle uncut during first half of experiment. Stimulation produced rise in arterial B. P. so long as pedicle uncut.
	0.233 (*15*)	0.243 (*3*)	4	
	0.292 (*20*)	0.372 (*8*)	27.3	
	0.425 (*14*)	0.472 (*6*)	11	

[1] See footnote to Table I.

Stimulation of the hepatic nerves with the adrenal glands removed. — With the adrenals removed from the circulation[19] (Table VI, Nos. 85 to 87), quite different results were obtained. No increase in the reducing power of the vena cava plasma was observed, even after stimulation for ten minutes with secondary coil at 4 cm. The first specimen of blood for analysis in each of the experiments was removed in from fifteen to thirty minutes after the adrenalectomy. The periods of time after stimulation at which the other specimens of blood were removed are indicated as usual by the figures in brackets. In two of

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[19] The adrenals were not really removed from the body, but were completely isolated by the application of at least three mass ligatures tightly tied under them at different places and including the veins. The complete isolation was verified by careful post-mortem examination. A little time is saved by this technique.

TABLE VI.

THE EFFECT ON THE REDUCING SUBSTANCE OF THE BLOOD PLASMA OF THE VENA CAVA INFERIOR PRODUCED BY STIMULATION OF THE HEPATIC NERVES AFTER REMOVAL OF BOTH ADRENAL GLANDS.

No of expt.	Per cent reducing substance.		Change produced.	Remarks.
	Before stimulat'n.	After stimulat'n.		
85	0.155	0.149 (*8*)[1]	Fall	Coil, 6 cm. Stimulation did not affect blood pressure.
	0.133 (*22*)	0.120 (*8*)	"	Blood pressure, 50 mm.[2]
86	0.323[3]	0.274 (*8*)	"	
	0.239 (*14*)	0.225 (*8*)	"	Blood pressure, 65 mm.
87	0.141[4]	0.145 (*9*)	No change	
	0.137 (*16*)	0.126 (*10*)	Fall	
88	0.124[5]	0.140 (*8*)	13	Blood used in first two, plasma in others. Animal very sensitive to ether.
	0.144[6]	0.133 (*8*)	Fall	
	....	0.110 (*25*)	"	
90	0.344[7]	0.291 (*10*)	"	
	0.230 (*20*)	0.234 (*10*)	No change	
91	0.156[5]	0.194 (*10*)	24	Coil, 4 cm. After the adrenalectomy the removal of blood caused a fall to 70 mm. Hg in blood pressure. The blood in cannula was also partly clotted. Adrenalin then injected causing blood sugar to rise to 0.387 per cent.
	0.273 (*43*)[6]	0.400 (*10*)	46	
92	0.179	0.157 (*10*)	Fall	

[1] The figures in Italic indicate the number of minutes elapsing between the removal of the various samples of blood. In order, the figures are to be read from left to right.

[2] Unless otherwise noted, the blood pressure was between 90–120 mm. Hg.

[3] Thirty minutes after adrenalectomy. Coil, 6 cm. Stimulation did not affect blood pressure.

[4] Thirty minutes after adrenalectomy. Coil, 4 cm. Stimulation did not affect blood pressure.

[5] Adrenals intact.

[6] Adrenals removed.

[7] High because splanchnic nerve had been stimulated earlier in expt. (see Table I).

the experiments (Nos. 88 and 91), the adrenals were left intact during the first part of the experiment. It is clear that their excision rendered the stimulation of the hepatic plexus incapable of producing hyperglycogenolysis. The results of one experiment (No. 91) are exactly the opposite to those of the other six. We believe that this is because of a very low blood pressure produced in this dog by removal of the third sample of blood. The blood also was partially clotted in the cannula and was very venous in color. We have invariably found that blood removed from the vena cava under these conditions has a high sugar content. The only conclusion which can be drawn from these results is that the presence of adrenalin in the blood is essential for the nerve control which is exercised over the glycogenolytic process by the hepatic nerves. We have little doubt that after adrenalectomy the hepatic nerves could be again rendered capable of controlling the glycogenolytic process by injecting adrenalin in the blood.

Langley[20] has postulated the presence in cells whose activities are under the control of the sympathetic system of two substances, — the chief substance, by the activities of which the cell performs its various functions, and the receptive substance, which exists between the chief substance and the sympathetic nerve ending. This receptive substance is supposed to be very readily damaged and to be the substance in the cell with which adrenalin combines. When it does so in excess, the receptive substance causes a stimulation of the chief substance, just as would be the case were the nerve fibres themselves stimulated. When adrenalin is absent from the blood, however, then it is probable that the receptive substance can no longer transmit the stimulus from the nerve endings to the chief substance; in other words, the functions which previously could be excited by stimulation of the nerve fail now to react in their usual manner.

We are not aware that the excitability of the various motor functions controlled by the sympathetic system has been studied in animals from which the adrenal capsules have been removed. We know, however, that some of these functions, such as the dilatation of the pupil and acceleration of the heart, can be produced by stimulation of the sympathetic fibres in artificially perfused preparations. The receptive substance of the glycogenolytic nerve control must therefore be more susceptible to the absence of adrenalin than is that of certain of the motor functions under sympathetic control.

Adrenalin does not directly affect the glycogenolytic activity of extracts of liver. This is shown by the fact that the percentile disappearance of glycogen from an incubated mixture of liver extract and glycogen solution is not in the least degree changed when even large amounts of fresh extract of the suprarenal gland are added to them.[21]

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[20] LANGLEY: Journal of physiology, 1905–1906, xxxiii, p. 400.

SUMMARY.

In the experiments recorded in this paper the amount of reducing substance of the blood, or blood plasma, removed from the vena cava opposite the liver was compared under such conditions as would demonstrate the relationship of the adrenal gland to the process of hepatic glycogenolysis. The following facts were elicited:

After excision of the left adrenal gland, stimulation of the great splanchnic nerve, on the same side, is not followed by the hyperglycogenolysis, which ordinarily follows the stimulation of this nerve.

The same result is usually obtained after ligation of the main adrenal veins on both sides. Occasionally, however, this is not the case, possibly because some smaller veins remain unligated.

These results do not indicate that it is on account of a hypersecretion of adrenalin into the blood that stimulation of the splanchnic nerves ordinarily causes hyperglycogenolysis, because, after complete section of the hepatic plexus, stimulation of the splanchnic nerve is only occasionally followed by an increase in the sugar content of the vena cava blood (hyperglycæmia).

Were hypersecretion of the adrenal gland the cause of the hyperglycæmia which follows stimulation of the splanchnic nerve, this hyperglycæmia should be as marked after section of the hepatic plexus as before.

Stimulation of the hepatic plexus, either cut or uncut, causes marked hyperglycæmia, provided the adrenal glands are intact. When the adrenal glands are removed, however, stimulation of the hepatic plexus does not cause hyperglycæmia.

Injection of adrenalin in the portal vein causes hyperglycæmia after the hepatic plexus is cut, indicating that it can excite the local glycogenolytic mechanism.

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[21] Allowance must of course be made for any possible glycogenolysis that the suprarenal extract may show by itself.

It is concluded that it is only when the adrenal glands are intact that it is possible, by stimulation of the nerves supplying the liver, to excite hyperglycogenolysis. Some influence exercised by the adrenal glands is evidently essential for the functional integrity of the nerves which control the process of glycogenolysis.

A SIMPLE METHOD FOR DETERMINING THE CARBON DIOXIDE CONTENT OF THE ALVEOLAR AIR BY MEANS OF BARYTA.

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THE method to be here described was devised primarily for the use of elementary students in a laboratory course of physiology. It has proved to be so easy and simple, requiring nothing in the way of apparatus other than common laboratory material, and yet withal so accurate, that it seems well suited not only for purposes of instruction but also for research. Its principal drawback is that it determines only CO_2, and not the oxygen also.

The refined and accurate gas analysis apparatus of Haldane has, in the hands of an expert who is using the instrument daily, no difficulties beyond those necessarily associated with any combination of ground glass stopcocks, mercury, and strong potash solution. In the hands of a beginner, however, or when the apparatus has not been used for some time, it has, like all such instruments, various disconcerting and even costly tricks. These the method to be here described avoids. Lest, however, we should seem to confuse a little thing with a great one, we hasten to add that our method is merely a slight modification of the epoch-making procedure of Haldane and Priestley.

The arrangement of apparatus which we have used is shown in the accompanying figure. It consists of (*A*) a piece of glass tubing 1 to 1.5 cm. in diameter and 5 to 8 cm. long. This serves as a mouthpiece to which the tongue can be applied after a deep expiration. It fits into (*B*) a piece of garden hose, or tubing of similar size, 1.5 to 2.5 metres in length. In the side of the hose, close to the mouthpiece, is a hole into which is inserted (*C*) a pipette. This is the only peculiar part of the apparatus. We have used for the purpose one of the triple-bulb splash tubes (having a total volume of about 150 c.c.) supplied with a Hill blood-gas pump. If such a tube is not available, a substitute is easily made from the bulbs of two pipettes, one twice as large as the other (*e. g.*, 100 c.c. and 50 c.c., or 50 c.c. and 25 or 20 c.c.). It is best to cut off the extra glass tubing on the pipettes and to connect them with a bit of rubber tubing as shown (by *D*) in the figure.

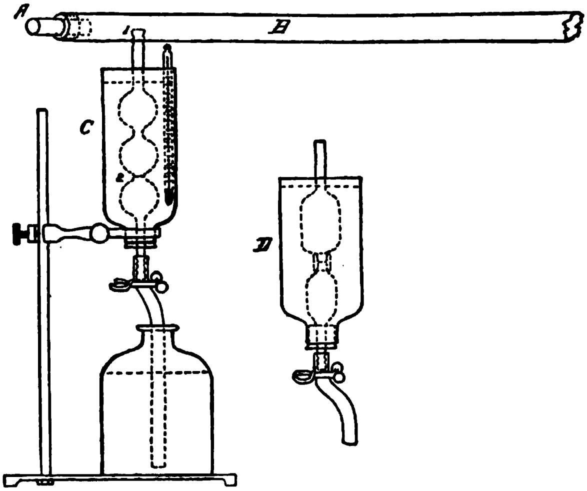

Figure showing arrangement of apparatus. For full description see text. *A*, mouthpiece; *B*, large rubber tube, or piece of garden hose; *C*, the air and baryta pipette; *D*, another form of the pipette. The level of the baryta before taking sample of air is marked 1. The level after drawing sample, by opening pinchcock and allowing escape of baryta, is marked 2.

On the lower end of the pipette is a rubber tube (30 to 50 cm. long) with a spring clip. Around the pipette is a water jacket in which is placed a thermometer. The jacket is conveniently made of a long narrow bottle with the bottom removed. This is done by wrapping a string moistened with alcohol around it and setting fire to the alcohol.

The volume of the two upper bulbs of the pipette must be determined, and also the volume of the lower bulb, up to a mark scratched with a file on the narrow tube between the middle and lower bulbs. This is done with sufficient accuracy by first drying the bulbs and then filling them with water from a burette. The solutions employed are a fortieth normal barium hydrate solution, and a fortieth normal sulphuric (or oxalic or hydrochloric) acid solution. It is well to add a small quantity of barium chloride to the baryta, — say 5 c.c. of 10 per cent barium chloride in each litre.

In preparing for an analysis the pipette is disconnected from the hose. The small rubber tube is lowered into the bottle of baryta water. The clip is opened. The entire pipette is sucked brimming full (to 1 in the figure). The clip is again closed upon the rubber tube as close as possible to the end of the pipette. The upper end of the pipette is then inserted through the hole in the side of the hose. The jacket is filled with water at room temperature. The subject, after making sure that he is breathing quite naturally, applies his lips to the mouthpiece, makes as deep an expiration as possible through the hose, and immediately places his tongue against the mouthpiece. Holding his tongue in this position for four or five seconds, he may breathe as nature dictates while the operator draws a sample of air. This is done by opening the clip on the rubber tube at the bottom of the pipette and allowing the baryta to run out rapidly until the two uppermost bulbs (or the one uppermost in the form shown as *D* in the figure) are empty. The outflow is stopped when the surface of the baryta is at the line (designated 2 in the figure) previously cut with a file on the constriction between the two lowermost bulbs. The lowest bulb is thus left full of baryta, *i. e.*, a known volume of known strength with which the CO_2 in the air drawn into the upper bulbs (also a known volume) is later to be absorbed.

Twenty seconds are allowed to elapse while the sample of air in the two upper bulbs takes the temperature of the water in the jacket. This temperature is then noted. The upper end of the pipette is withdrawn from the hole in the hose, and a finger is instantly placed over its end. It is now inverted so as to pour the water out of the jacket and to allow the baryta to run down into the two empty bulbs. The entire pipette is then shaken vigorously for one minute. All of the CO_2 in the air which it contains is thus combined with the baryta and precipitated as $BaCO_3$. The rubber tube is then slipped off of the end of the pipette without opening the pinchcock. The now milky baryta is run off into a small dry flask, which is immediately stoppered and set aside.

After a couple of hours sedimentation is complete. Now 10 c.c. of the clear supernatant fluid are pipetted off into a small Erlenmeyer flask, and titrated with the fortieth normal acid. Phenolphthalein is used as the indicator. The barometer is read; the tension of water vapor at the observed temperature of the water jacket is subtracted from the barometric reading; and the weight of 1 c.c. of pure CO_2 at the observed temperature and at this corrected pressure is taken from a table (to be found in any chemical handbook). From the titration and the volumes of the upper and lower parts of the pipette we calculate the actual weight of carbon dioxide in 1 c.c. of the sample of alveolar air. This divided by the weight of 1 c.c. of pure CO_2 (as above) gives the percentage of CO_2 which was contained in the dry alveolar air of the lungs. This percentage multiplied by the barometric reading minus 47 mm. (water vapor in the lungs at body temperature) gives the partial pressure of CO_2 in millimetres of mercury in the alveolar air.

From the foregoing account it will be seen that we have employed exactly the same procedure for obtaining alveolar air as do Haldane and his collaborators. We have likewise taken as the correct figure the mean of two determinations — one inspiratory, the other expiratory. This means that the quick deep expiration (a sort of artificial cough) is made in the one case immediately after (*i. e.*, at the height of) a natural inspiration, and in the other after a natural expiration. Herein lie the principal difficulty and source of error. Many persons are inclined at the first trial to draw an extra deep breath before making the expiration through the tube. As a result the CO_2 comes out too low. When warned of this mistake, they may breathe subnormally. The sample of air is then found to have an abnormally high percentage of CO_2.

With all except unusually "nervous" persons it is sufficient to explain these points, and to have the subject make two or three preliminary trials at expiring through the tube, — a matter of only a couple of minutes or less when no sample is withdrawn for analysis. Then, when the samples are taken, they afford on analysis figures which are usually correct within one or two tenths of 1 per cent. Thus five successive "inspiratory" samples (from D. G. R.) gave the following figures:

5.47, 5.51, 5.59, 5.50, 5.64, — average, 5.54.

Determinations of both inspiratory and expiratory samples by means of the Haldane apparatus and by the baryta method showed:

Subject.		With Haldane apparatus.	By baryta method.
Y. H.	Inspiratory sample	5.09	5.09
	Expiratory "	5.25	5.18
	Mean "	5.17	5.14
D. G. R.	Inspiratory "	5.54	5.48
	Expiratory "	5.77	5.64
	Mean "	5.65	5.56

We have used this method satisfactorily with a class of students to determine not only the CO_2 during rest, but also after exercise (running upstairs) and at the "breaking point" of holding the breath. Our results tend to confirm the view (if indeed additional confirmation is needed) that what we may term the *capnicity* of the body is automatically maintained with a precision equalling that exhibited in the regulation of body temperature. From this we may fairly infer that one is as essential a condition of health as is the other.

AN EXPLANATION OF HUNGER.[1]

By W. B. CANNON and A. L. WASHBURN.

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HUNGER and appetite are so intimately interrelated that a discussion of either requires each to be clearly defined. According to one view the two experiences differ only quantitatively, appetite being a mild stage of hunger.[2] Another view, better supported by observations, is that the two experiences are fundamentally different.[3]

Appetite is related to previous sensations of the taste and smell of food; it has therefore, as Pawlow has shown, important psychic elements. It may exist separate from hunger, as, for example, when we eat delectable dainties merely to please the palate. Sensory associations, delightful or disgusting, determine the appetite for any edible substance, and either memory or present stimulation can thus arouse desire or dislike for food.

Hunger, on the other hand, is a dull ache or gnawing sensation referred to the lower mid-chest region and the epigastrium. It is the organism's first strong demand for nutriment, and, not satisfied, is likely to grow into a highly uncomfortable pang, less definitely localized as it becomes more intense. It may exist separate from appetite, as, for example, when hunger forces the taking of food not only distasteful but even nauseating. Besides the dull ache, however, lassitude and drowsiness may appear, or faintness, or headache, or irritability and restlessness such that continuous effort in ordinary affairs becomes increasingly difficult. That these states differ with individuals — headache in one, faintness in another, for example — indicates that they do not constitute the central fact of hunger, but are more or less inconstant accompaniments, for the present negligible. The dull, pressing sensation is the constant characteristic, the central fact, to be examined in detail.

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[1] The indicative evidence of the results here reported was presented to the Boston Society of Medical Sciences, January 17, 1911. See CANNON: The mechanical factors of digestion, London and New York, 1911, p. 204. The full account was presented to the Harvey Society, New York City, December 16, 1911.

[2] BARDIER: Richet's Dictionnaire de physiologie, article "Faim," 1904, vi, p. 1; HOWELL: Text-book of physiology, fourth edition, Philadelphia and London, 1911, p. 285.

[3] See STERNBERG: Zentralblatt für Physiologie, 1909, xxii, p. 653. Similar views were expressed by BAYLE in a thesis presented to the Faculty of Medicine in Paris in 1816.

Of the two theories of hunger — (1) that it is a general sensation with a local reference, and (2) that it has a local peripheral source — the former has been more widely accepted. The support for that theory can be shown to be not substantial. The wide acceptance of the theory, however, warrants an examination of it in some detail.

HUNGER NOT A GENERAL SENSATION.

Underlying the idea that hunger arises from a general condition of the body is the consideration that, as time passes, food substances disappear from the blood, and consequently the nerve cells, suffering from the shortage of provisions, give rise to the sensation.[4]

In support of this view the increase of hunger as time passes has been pointed out. There is abundant evidence, however, that the period of increase is short, and that during continued fasting hunger wholly disappears after the first few days.[5] On the theory that hunger is a manifestation of bodily need, we must suppose that the body is mysteriously not in need after the third day, and that therefore hunger disappears. The absurdity of such a view is obvious.

Continued hunger soon after eating (when the stomach is full), especially in cases of duodenal fistula,[6] and satisfaction when the escaping chyme is restored to the intestine, have been cited as ruling out the peripheral and thus favoring the central origin of the sensation. As will be seen later, however, other possible peripheral sources of hunger exist besides the stomach. Further consideration of this point will be given in due course.

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[4] SCHIFF: Physiologie de la digestion, Florence and Turin, 1867, p. 40.

[5] See LUCIANI: Das Hungern, Hamburg and Leipzig, 1890, p. 113; TIGERSTEDT: Nagel's Handbuch der Physiologie, Berlin, 1909, i, p. 376; JOHANSSON, LANDERGREN, SONDÉN, and TIGERSTEDT: Skandinavisches Archiv für Physiologie, 1897, vii, p. 33; CARRINGTON: Vitality, fasting and nutrition, New York, 1908, p. 555; VITERBI: quoted by BARDIER, *Loc. cit.*, p. 7.

[6] See BUSCH: Archiv für pathologische Anatomie und Physiologie und für klinische Medicin, 1858, xiv, p. 147.

Because animals eat, sometimes eagerly, when the gastro-intestinal tract is wholly separated from the central nervous system,[7] the conclusion has been drawn that hunger must be a general sensation and not of peripheral origin. But appetite as well as hunger may lead to eating. As Ludwig stated many years ago, even if all afferent nerves were severed, psychic reasons still could be given for the taking of food.[8] Indeed, who accepts dessert because he is hungry? Evidently, since hunger is not required for eating, the fact that an animal eats is no testimony whatever that the animal is hungry, and therefore, after nerves have been severed, is no proof that hunger is of central origin.

Further objections to the theory that hunger is a general sensation lie in the weakness of its main assumption and in its failure to account for certain well-known observations. Thus no evidence exists that the blood has in fact changed when hunger appears. Moreover in fever, when bodily stores are being most rapidly destroyed, and when therefore, according to this theory, hunger should be most insistent, the sensation is wholly absent. And the quick abolition of the pangs soon after food is taken, before digestion and absorption can have proceeded far, as well as the quieting effect of swallowing indigestible stuff, such as moss and clay, further weakens the argument that the sensation arises directly from lack of nutriment in the body.

Many have noted that hunger has a sharp onset. If this abrupt arrival of the characteristic ache corresponds to the general bodily state, the change in general bodily state must occur with like suddenness, or have a critical point at which the sensation is instantly precipitated. No evidence exists that either of these conditions occurs in metabolism.

Another peculiarity of hunger which we have noticed is its intermittency. It may come and go several times in the course of a few hours. Furthermore, during a given period, the sensation is not uniform in intensity, but is marked by ups and downs, sometimes changing to alternate presence and absence without alteration of rate. Our observations have been confirmed by psychologists, trained to introspection, who have reported that the sensation has a distinctly intermittent course.[9] In the experience of one of us (C.) the hunger pangs came and went on one occasion as follows:

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[7] See SCHIFF: *Loc. cit.*, p. 37; also DUCCESCHI: Archivio di fisiologia, 1910, viii, p. 582.

[8] LUDWIG: Lehrbuch der Physiologie des Menschen, Leipzig and Heidelberg, 1858, ii, p. 584.

Came	Went
12–37–20	12–38–30
40–45	41–10
41–45	42–25
43–20	43–35
44–40	45–55
46–15	46–30

and so on, for ten minutes longer. Again in this relation, the intermittent and periodic character of hunger would require, on the central theory, that the bodily supplies be intermittently and periodically insufficient. During one moment absence of hunger would imply abundance of nutriment in the organism, ten seconds later presence of hunger would imply that the stores had been suddenly reduced, ten seconds later still absence of hunger would imply sudden renewal of plenty. Such zigzag shifts of the general bodily state may not be impossible, but, from all that is known of the course of metabolism, they are highly improbable. The periodicity of hunger, therefore, is further evidence against the theory that the sensation has a general basis in the body.

The last objection to this theory is its failure to account for the most common feature of hunger, — the reference of the sensation to the epigastric region. Schiff and others[10] have met this objection by two contentions. First, they have pointed out that hunger is not always referred to the stomach. Schiff interrogated ignorant soldiers regarding the local reference; several indicated the neck or chest, twenty-three the sternum, four were uncertain of any region, and two only designated the stomach. In other words, the stomach region was most rarely mentioned.

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[9] We are indebted to Prof. J. W. Baird of Clark University, and his collaborators, for this corroborative testimony.

[10] See Schiff: *Loc. cit.*, p. 31; Bardier: *Loc. cit.*, p. 16.

The second contention against the importance of local reference is that such evidence is fallacious. Just as the reference of tinglings to fingers which have been removed from the body does not prove that the tinglings originate in those fingers, so the assignment of the ache of hunger to any special region does not demonstrate that the ache arises from that region.

Concerning these arguments we may recall first Schiff's admission that the soldiers he questioned were too few to give conclusive evidence. Further, the testimony of most of them that hunger seemed to originate in the region of the sternum cannot be claimed as unfavorable to a peripheral source of the sensation. The description of feelings which develop from disturbances within the body is almost always indefinite; the testimony is not, therefore, dismissed as worthless. On the contrary, such testimony is used constantly in judging internal disorders.

The force of the contention that reference to the periphery is not proof of the peripheral origin of a sensation depends on the amount of accessory evidence which is available. Thus, if an object is seen coming into contact with a finger, the simultaneous sensation of touch referred to that finger may reasonably be assumed to have resulted from the contact, and not to have been a purely central experience accidentally attributed to an outlying member. Similarly in the case of hunger — all that is needed as support for the peripheral reference of the sensation is proof that conditions occur there, simultaneously with hunger pangs, which might reasonably be regarded as giving rise to those pangs. In the fasting stomach may not conditions, in fact, be present which would sustain the theory that hunger has a local peripheral source?

Certain assumptions have been made regarding the state of the fasting stomach, and certain inferences have been drawn from these assumptions which must be considered before the results we have to present will have a proper setting.

OBJECTIONS TO SOME THEORIES THAT HUNGER IS OF LOCAL ORIGIN.

Hunger is not due to emptiness of the stomach, for Nicolai found after gastric lavage that the sensation did not appear in some instances for more than three hours.[11] This testimony confirms Beaumont's observation on Alexis St. Martin, that hunger arises some time after the stomach is evacuated.[12]

Hunger is not due to hydrochloric acid secreted into the empty stomach. The gastric wash-water from hungry subjects is neutral or only slightly acid.[13] Furthermore, persons suffering from achylia gastrica declare that they have normal feelings of hunger.

Hunger is not due to turgescence of the gastric glands. This theory, propounded by Beaumont,[14] has commended itself to several recent writers. Thus Luciani has accepted it, and by adding the idea that nerves distributed to the mucosa are specially sensitive to deprivation of food, he accounts for the hunger pangs.[15] Also Valenti declared two years ago that the turgescence theory of Beaumont is the only one possessing a semblance of truth.[16] The experimental work reported by these two investigators, however, does not necessarily support the turgescence theory. Luciani severed in fasting dogs the previously exposed and cocainized vagi, and Valenti merely cocainized the nerves; the dogs, eager to eat a few minutes previous to this operation, now ran about as before, but when offered food, licked and smelled it, but did not take it. This total neglect of the food lasted varying periods up to two hours. The vagus nerves seem, indeed, to convey impulses which affect the procedure of eating, but there is no clear evidence that those impulses arise from distention of the gland cells. The turgescence theory would also meet difficulties in an attempt to explain the disappearance of hunger after the swallowing of indigestible material; for such material, not being appetizing, does not cause any secretion of gastric juice.[17] Furthermore, Nicolai found that the sensation could be abolished by simply introducing a stomach tube. The turgescence of the gastric glands would not be reduced by either of these procedures. The turgescence theory, finally, does not explain the quick onset of hunger, or its intermittent and periodic character, for the cells cannot be repeatedly swollen and contracted within periods a few seconds in duration.

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[11] NICOLAI: Ueber die Entstehung des Hungergefühls, Inaugural-Dissertation, Berlin, 1892, p. 17.

[12] BEAUMONT: The physiology of digestion, second edition, Burlington, 1847, p. 51.

[13] NICOLAI: *Loc. cit.*, p. 15.

[14] BEAUMONT: *Loc. cit.*, p. 55.

[15] LUCIANI: Archivio di fisiologia, 1906, iii, p. 542.

[16] VALENTI: Archives italiennes de biologie, 1910, liii, p. 97.

[17] See PAWLOW: The work of the digestive glands, London, 1902, p. 70; HORNBORG: Skandinavisches Archiv für Physiologie, 1904, xv, p. 248.

HUNGER THE RESULT OF CONTRACTIONS.

There remain to be considered, as a possible cause of hunger pangs, contractions of the stomach and other parts of the alimentary canal. This suggestion is not new. Sixty-six years ago Weber declared his belief that "strong contraction of the muscle fibres of the wholly empty stomach, whereby its cavity disappears, makes a part of the sensation which we call hunger."[18] Vierordt drew the same inference twenty-five years later (in 1871);[19] and since then Knapp and also Hertz have declared their adherence to this view. These writers have not brought forward any direct evidence for their conclusion, though Hertz has cited Boldireff's observations on fasting dogs as probably accounting for what he terms "the gastric constituent of the sensation."[20]

The argument commonly used against the contraction theory is that the stomach is not energetically active when empty. Thus Schiff stated "the movements of the empty stomach are rare and much less energetic than during digestion."[21] Luciani expressed his disbelief by asserting that gastric movements are much more active during gastric digestion than at other times, and cease almost entirely when the stomach has discharged its contents.[22] And Valenti stated only year before last: "We know very well that gastric movements are exaggerated while digestion is proceeding in the stomach, but when the organ is empty they are more rare and much less pronounced," and therefore they cannot account for hunger.[23]

Contractions of the alimentary canal in fasting animals. — Evidence opposed to these suppositions has been in existence for many years.

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[18] WEBER: Wagner's Handwörterbuch der Physiologie, 1846, iii², p. 580.

[19] VIERORDT: Grundriss der Physiologie, Tübingen, 1871, p. 433.

[20] KNAPP: American medicine, 1905, x, p. 358; HERTZ: The sensibility of the alimentary canal, London, 1911, p. 37.

[21] SCHIFF: *Loc. cit.*, p. 33.

[22] LUCIANI: *Loc. cit.*, p. 542.

[23] VALENTI: *Loc. cit.*, p. 97.

In 1899 Bettmann called attention to the contracted condition of the stomach after several days' fast.[24] In 1902 Wolff reported that after forty-eight hours without food the stomach of the cat may be so small as to look like a slightly enlarged duodenum.[25] The anatomist His has also observed the phenomenon.[26] Seven years ago Boldireff demonstrated that the whole gastro-intestinal tract has a periodic activity while not digesting.[27] Each period of activity lasts from twenty to thirty minutes, and is characterized in the stomach by rhythmic contractions 10 to 20 in number. These contractions, Boldireff reports, may be stronger than during digestion, and his published records clearly support this statement. The intervals of repose between periodic recurrences of the contractions last from one and a half to two and a half hours. Especially noteworthy is Boldireff's observation that if fasting is continued for two or three days the groups of contractions appear at gradually longer intervals and last for gradually shorter periods, and thereupon the gastric glands begin continuous secretion, and all movements cease. All these testimonies to increased tone and periodic pulsations definitely prove, contrary to previous statements, that the empty stomach may be the seat of vigorous muscular activities.

Boldireff considered hunger in relation to the activities he described, but solely with the idea that hunger might *provoke* them; and since the activities dwindled in force and frequence as time passed, whereas in his belief they should have become more pronounced, he abandoned the notion of any relation between the phenomena.[28] Did not Boldireff misinterpret his own observations? When he was considering whether hunger might cause the contractions, did he not overlook the possibility that the contractions might cause hunger? A number of experiences have led to the conviction that Boldireff did, indeed, fail to perceive part of the significance of his results. For example, in auscultation of the alimentary canal relatively loud borborygmi have been noted as the hunger pangs were disappearing. Again the sensation can be momentarily abolished a few seconds after swallowing a small accumulation of saliva or a tablespoonful of water. Since the stomach is in high tonus in hunger, this result can be accounted for as due to the momentary inhibition of the tonus by swallowing.[29] Thus also could be explained the disappearance of the ache soon after eating is begun, for repeated swallowing results in continued inhibition.

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[24] Bettmann: Philadelphia monthly medical journal, 1899, i, p. 133.

[25] Wolff: Dissertation, Giessen, 1902, p. 9.

[26] His: Archiv für Anatomie, 1903, p. 345.

[27] Boldireff: Archives biologiques de St. Petersburg, 1905, xi, p. 1. See also Ergebnisse der Physiologie, 1911, xi, p. 182.

[28] Boldireff: *Loc. cit.*, p. 96.

The concomitance of contractions and hunger in man. — Although the evidence above mentioned had led to the conviction that hunger results from contractions of the alimentary canal, direct proof was still lacking. In order to learn whether such proof might be secured, one of us (W.) determined to become accustomed to the presence of a rubber tube in the œsophagus.[30] Almost every day for several weeks W. introduced as far as the stomach a small tube, to the lower end of which was attached a soft-rubber balloon about 8 cm. in diameter. The tube was thus carried about each time for two or three hours. After this preliminary experience the introduction of the tube, and its presence in the gullet and stomach, were not at all disturbing. When a record was to be taken, the balloon, placed just below the cardia, was moderately distended with air, and was connected with a water manometer ending in a cylindrical chamber 3.5 cm. wide. A float recorder resting on the water in the chamber permitted registering any contractions of the fundus of the stomach. On the days of observation W. would abstain from breakfast, or eat sparingly, and without taking any luncheon would appear in the laboratory about two o'clock. The recording apparatus was arranged as above described. In order to avoid the possibility of an artifact, a pneumograph, fastened below the ribs, was made to record the movements of the abdominal wall. Between the records of gastric pressure and abdominal movement one electromagnetic signal marked time in minutes, and another traced a line which could be altered by pressing a key. All these recording arrangements were out of W's sight; he sat with one hand at the key, ready, whenever the sensation of hunger was experienced, to make the current which moved the signal.

When W. stated that he was hungry, powerful contractions of the stomach were invariably being registered. The record of W's introspection of his hunger pangs agreed closely with the record of his gastric contractions. Almost invariably, however, the contraction nearly reached its maximum before the record of the sensation was started (see Fig. 1). This fact may be regarded as evidence that the contraction precedes the sensation, and not *vice versa*, as Boldireff considered it. The contractions were about a half-minute in duration, and the intervals between varied from thirty to ninety seconds, with an average of about one minute. W's augmentations of intragastric pressure ranged between 11 and 13 in twenty minutes; C. had previously counted in himself 11 hunger pangs in the same time (see ten-minute record, p. 444). The rate in each of us, therefore, proved to be approximately the same. This rate is slightly slower than that found in dogs by Boldireff; the difference is perhaps correlated with the slower rhythm of gastric peristalsis in man compared with that in the dog.[31]

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[29] CANNON and LIEB: this Journal, 1911, xxix, p. 267.

[30] NICOLAI (*Loc. cit.*) reported that although the introduction of a stomach tube at first abolished hunger in his subjects, with repeated use the effects became insignificant.

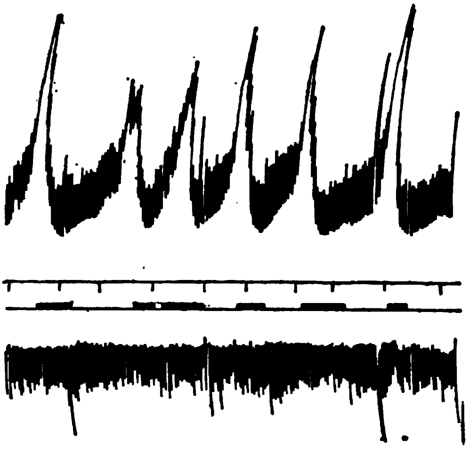

FIGURE 1. — One half the original size. The top record represents intragastric pressure (the small oscillations due to respiration, the large to contractions of the stomach); the second record is time in minutes (ten minutes); the third record is W's report of hunger pangs; the lowest record is respiration registered by means of a pneumograph about the abdomen.

Before hunger was experienced by W. the recording apparatus revealed no signs of gastric activity. Sometimes a rather tedious period of waiting had to be endured before contractions occurred, and after they began they continued for a while, then ceased (see Fig. 2). The feeling of hunger, which was reported while the contractions were recurring, disappeared when they stopped. The inability of the subject to control the contractions eliminated the possibility of their being artifacts, perhaps induced by suggestion. The close concomitance of the contractions with hunger pangs, therefore, clearly indicates that they are the real source of those pangs.

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[31] CANNON: The mechanical factors of digestion, London and New York, 1911, p. 54.

Boldireff's studies proved that when the empty stomach is manifesting periodic contractions the intestines also are active. Conceivably all parts of the alimentary canal composed of smooth muscle share in these movements. The lower œsophagus in man is provided with smooth muscle. It was possible to determine whether this region in W. was active during hunger.

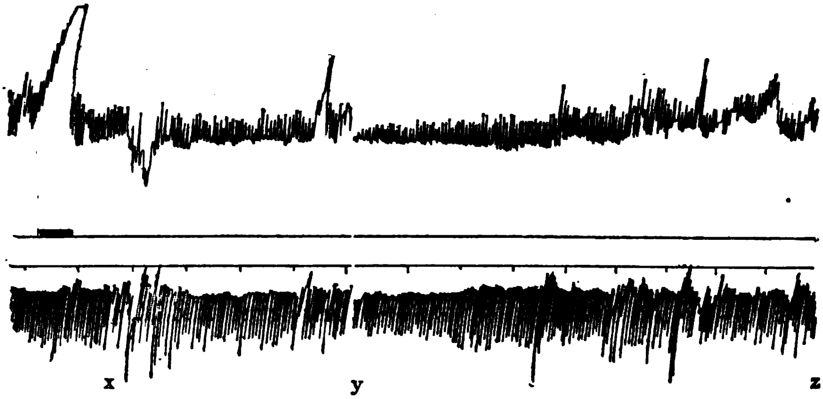

FIGURE 2. — One half the original size. The same conditions as in Fig. 1 (fifteen minutes). There was a long wait for hunger to disappear. After *x*, W. reported himself "tired, but not hungry." The record from *y* to *z* was the continuance, on a second drum, of *x* to *y*.

To the œsophageal tube a thin-rubber finger cot (2 cm. in length) was attached and lowered into the stomach. The little rubber bag was distended with air, and the tube, pinched to keep the bag inflated, was gently withdrawn until resistance was felt. The air was now released from the bag, and the tube further withdrawn about 3 cm. The bag was again distended with air at a manometric pressure of 10 cm. of water. Inspiration now caused the writing lever, which recorded the pressure changes, to rise; and a slightly further withdrawal of the tube changed the rise, on inspiration, to a fall. The former position of the tube, therefore, was above the gastric cavity and below the diaphragm. In this position the bag, attached to a float recorder (with chamber 2.3 cm. in diameter) registered the periodic oscillations shown in Fig. 3. Though individually more prolonged than those of the stomach, these contractions, it will be noted, occur at about the same rate. It is probable that the periodic activity of the two regions is simultaneous, for otherwise the stomach would force its gaseous content into the œsophagus with the rise of intragastric pressure.

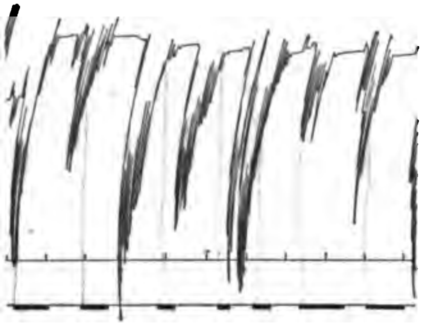

FIGURE 3. — One half the original size. The top record represents compression of a thin-rubber bag in the lower œsophagus. The pressure in the bag varied between 9 and 13 cm. of water. The cylinder of the recorder was of smaller diameter than that used in the gastric records. The œsophageal contractions compressed the bag so completely that, at the summits of the large oscillations, the respirations were not registered. When the oscillations dropped to the time line, the bag was about half inflated. The middle line registers time in minutes (ten minutes). The bottom record is W's report of hunger pangs.

What causes the contractions to occur has not been determined. From evidence already given they do not seem to be directly related to bodily need. Habit no doubt plays an important rôle. For present considerations, however, it is enough that they do occur, and that they are abolished when food, which satisfies bodily need, is taken into the stomach. By such indirection are performed some of the most fundamental of the bodily functions.

Peculiarities of hunger explained by contractions. — If these contractions are admitted as the cause of hunger, most of the difficulties confronting other explanations are readily obviated. Thus the occurrence of hunger at meal-times is most natural, for, as the regularity of defecation indicates, the alimentary canal has habits. Activity returns at the usual meal-time as the result of custom. By taking food regularly at a definite hour in the evening for several days, a new hunger period can be established. Since at these times the empty stomach, as Boldireff showed, has stronger contractions than the filled organ, hunger is aroused.

The contractions furthermore explain the sudden onset of hunger and its peculiar periodicity — phenomena which no other explanation of hunger can account for. The quick development of the sensation after taking a cold drink is possibly associated with the well-known power of cold to induce contraction in smooth muscle.

The great intensity of hunger during the first day of starvation, and its gradual disappearance till it vanishes on the third or fourth day, are made quite clear, for Boldireff observed that gastric contractions in his fasting dogs went through precisely such alterations of intensity and were not seen after the third day.

In fever, when bodily material is being most rapidly used, hunger is absent. Its absence is understood from an observation reported four years ago, that infection with systemic involvement is accompanied by a total cessation of all movements of the alimentary canal.[32] Boldireff observed that when his dogs were fatigued the rhythmic contractions failed to appear. Being "too tired to eat" is thereby given a rational explanation.

Another pathological form of the sensation — the inordinate hunger (bulimia) of certain neurotics — is in accord with the well-known disturbances of the tonic innervation of the alimentary canal in such individuals.

Since the lower end of the œsophagus, as well as the stomach, contracts periodically in hunger, the reference of the sensation to the sternum by the ignorant persons questioned by Schiff was wholly natural. The activity of the lower œsophagus also explains why, after the stomach has been removed, or in some cases when the stomach is distended with food, hunger can still be experienced. Conceivably the intestines also originate vague sensations by their contractions. Indeed the final banishment of the modified hunger sensation in the patient with duodenal fistula, described by Busch, may have been due to the lessened activity of the intestines when chyme was injected into them.

The observations recorded in this paper have, as already noted, numerous points of similarity to Boldireff's observations on the periodic activity of the alimentary canal in fasting dogs. Each period of activity, he found, comprised not only widespread contractions of the digestive canal, but also the pouring out of bile, and of pancreatic and intestinal juices rich in ferments. Gastric juice was not secreted at these times; when it was secreted and reached the intestine, the periodic activity ceased.[33] What is the significance of this extensive disturbance? Recently evidence has been presented that gastric peristalsis is dependent on the stretching of gastric muscle when tonically contracted.[34] The evidence that the stomach is in fact strongly contracted in hunger — *i. e.*, in a state of high tone — has been presented above.[35] Thus the very condition which causes hunger and leads to the taking of food is the condition, when the swallowed food stretches the shortened muscles, for immediate starting of gastric peristalsis. In this connection the recent observations of Haudek and Stigler are probably significant. They found that the stomach discharges its contents more rapidly if food is eaten in hunger than if not so eaten.[36] Hunger, in other words, is normally the signal that the stomach is contracted for action; the unpleasantness of hunger leads to eating; eating starts gastric secretion, distends the contracted organ, initiates the movements of gastric digestion, and abolishes the sensation. Meanwhile pancreatic and intestinal juices, as well as bile, have been prepared in the duodenum to receive the oncoming chyme. The periodic activity of the alimentary canal in fasting, therefore, is not solely the source of hunger pangs, but is at the same time an exhibition in the digestive organs of readiness for prompt attack on the food swallowed by the hungry animal.

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[32] CANNON and MURPHY: Journal of the American Medical Association, 1907, xlix, p. 840.

[33] BOLDIREFF: *Loc. cit.*, pp. 108–111.

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[34] CANNON: this Journal, 1911, xxix, p. 250.

[35] The "empty" stomach and œsophagus contain gas (see HERTZ: Quarterly journal of medicine, 1910, iii, p. 378; MIKULICZ: Mittheilungen aus dem Grenzgebieten der Medicin und Chirurgie, 1903, xii, p. 596). They would naturally manifest rhythmic contractions on shortening tonically on their content.

[36] HAUDEK and STIGLER: Archiv für die gesammte Physiologie, 1910, cxxxiii, p. 159.

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